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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **326th Meeting** *of the Biochemical Society was held in the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.* 1, *on Saturday,* 20 *February* 1954, *at* 11 *a.m. and took the form of a Symposium on:*

'THE CHEMICAL PATHOLOGY OF ANIMAL PIGMENTS'

COMMUNICATIONS

Normal and Pathological Metabolism of Porphyrins. By HELEN M. MUIR. (*Medical Unit, St Mary's Hospital Medical School, London, W.* 2)

Free porphyrins are usually present in the body only in microgram quantities, although the haem proteins contain relatively large amounts of porphyrins as metal complexes; these are all derivatives of protoporphyrin IX. (Protoporphyrin in proteins of 70 kg. man: 30·7 g. haemoglobin; 1·17 g. myoglobin; Drabkin, 1950.) No abnormal porphyrins have been demonstrated in protein complexes in diseased states, only as free porphyrins. Synthesis of porphyrin and protein moieties of haemoglobin are independent even in normal conditions; thus blood abnormalities do not necessarily imply abnormal porphyrin metabolism.

Breakdown to bile pigments of haem in haemoglobin is efficient and no significant re-utilization of haem can be demonstrated. There is no evidence to suggest that porphyrins other than protoporphyrin are catabolized to bile pigments; thus other porphyrins must be eliminated intact. Results of isotope studies of porphyrins and experimental porphyrinurias have elucidated the biochemical interrelationship of porphyrins. Type I porphyrins appear as by-products in the synthesis of protoporphyrin. All porphyrins are considered to be formed from a common monopyrrolic precursor, which may be porphobilinogen, recently isolated by Westall (1952), the structure of which has been determined by Cookson & Rimington (1953). Abnormalities of porphyrin metabolism will be discussed in the light of current views on the biosynthesis of porphyrins. Evidence will be presented from isotope studies and examination of free porphyrins in the tissues. Pharmacological effects of porphyrins will also be discussed. Synthesis of protoporphyrin in myoglobin, cytochrome and catalase takes place *in situ*, independently of medullary synthesis. Disturbances of porphyrin metabolism are not necessarily confined to the bone marrow.

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Patterns of Porphyrin Excretion. By J. E. FALK. (*Department of Chemical Pathology, University College Hospital Medical School, London, W.C.* 1)

The patterns of excretion of porphyrins in health and disease have long been studied, but only recently have the details of some underlying mechanisms begun to become clear. In addition, newer techniques have revealed unsuspected complexity in the nature of the porphyrins excreted. The paper chromatographic method of Nicholas & Rimington (1951) has shown the occurrence of minor amounts of porphyrins intermediate between the known octa-, tetra- and di-carboxylic porphyrins. It has long been recognized that both the series I and III isomers of uro- and copro-porphyrins occur naturally. Recent chromatographic methods (Falk & Benson, 1953; Chu, Green & Chu, 1951) have facilitated the isomer-analysis of these porphyrins.

With the isolation of porphobilinogen (Westall, 1952) and the elucidation of its structure (Cookson & Rimington, 1953; Cookson, 1953), a further complication has arisen. This monopyrrole is probably an important physiological precursor of uro-, copro- and proto-porphyrins (Falk, Dresel & Rimington, 1953), but in addition it is readily converted *in vitro* to material which behaves like a mixture of uroporphyrins I and III (cf. Cookson & Rimington, 1954). No paper chromatographic method has yet revealed more than two substances in these mixtures, though in the absence of enzymic control uroporphyrins II and IV theoretically should be present as well. The existing methods thus have serious limitations, and the possible occurrence of porphyrins of isomeric series II and IV, even in physiological material, can no longer be

dismissed. The provision of methods for their detection constitutes one of the most urgent problems in this field.

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Pathology of Acute Porphyria. Experimental Porphyria in Animals. By A. GOLDBERG. (*Department of Chemical Pathology, University College Hospital Medical School, London, W.C.* 1)

The pathological histology of autopsy tissues in acute porphyria generally shows variable changes in liver, kidney and nervous system (Prunty, 1946, 1949; Denny-Brown & Sciarra, 1945). Further evidence is presented that the nervous lesion involves myelin degeneration. The chemical examination of autopsy tissues emphasizes that the liver and sometimes the kidney and plasma are the only tissues containing porphobilinogen, the excretion of which is the essential metabolic abnormality in acute porphyria.

Investigations have been carried out to find the best method of inducing experimental 'porphyria' in animals. Sedormid (allyl*iso*propylacetylurea) (Schmid & Schwartz, 1952) is effective, but strongly hypnotic. The non-hypnotic allyl*iso*propylacetamide has been found to be a useful drug in producing this condition in rabbits (Goldberg, 1953). About 33 % of these animals produce large amounts of porphobilinogen and uroporphyrin for 1–3 weeks with no haematological changes and without the development of paralyses. They lose appetite, weight, and are constipated and may die from infection or renal damage, but their livers remain histologically normal and contain porphobilinogen at autopsy. The only change in urinary amino acids is a greatly diminished glycine excretion. Another 33 % of the animals produce only small amounts of porphobilinogen and uroporphyrin and eventually die with hepatic damage and an abnormal urinary amino-acid excretion. The livers of these animals contain no porphobilinogen at autopsy.

An experimental porphyria has also been induced in fowls. The porphyrins and porphobilinogen appear to be pharmacologically inactive (Goldberg, Paton & Thompson, 1954). The effect of certain barbiturates on the porphyrin metabolism of rabbits has been studied. Those containing an allyl group are particularly effective (Goldberg, 1954).

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The Chemical Pathology of Bile Pigments. 1. The Plasma Bile Pigments. By G. H. LATHE. (*The Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, London, W.* 6)

It is widely accepted that haemoglobin is broken down by cells of the reticulo-endothelial system and that bilirubin is released and becomes attached to plasma proteins. Plasma bile pigments are estimated colorimetrically by coupling with diazotized sulphanilic acid in acid solution. In this reaction bilirubin requires the presence of ethanol (or some other substances) and is said to give an 'indirect' reaction.

Circulating bilirubin is taken up by the liver and transformed into two pigments (I and II) which react 'directly', that is, without ethanol, in the diazo test. The more polar component, pigment II, appears to be the predominant pigment of bile. If the flow of bile is obstructed pigments I and II appear in the blood, attached to proteins, and in excess combine with other body constituents, producing jaundice. They do not pass into the brain.

The capacity of the normal adult liver to transform bilirubin is very great, and the plasma content of bilirubin remains relatively low, even with increased blood destruction. In the newborn baby, however, the bilirubin content of the plasma is elevated, and it appears that the ability of the liver

to transform bilirubin is limited. Should increased blood destruction also occur, as in haemolytic disease of the newborn, the plasma bilirubin content may become very high. Bilirubin is then taken up by other body structures, and these may include the brain.

Bilirubin pigmentation of the brain of the newborn baby is associated with brain damage, and death may ensue. A similar condition can be reproduced in experimental animals by the intravenous administration of large amounts of bilirubin.

The Chemical Pathology of Bile Pigments. 2. Bilirubinoid Pigments of Urine and Faeces and their Relation to Haem Pigment Metabolism. By C. H. Gray. (*Department of Chemical Pathology, King's College Hospital Medical School, London, S.E. 5*)

The presence of bilirubin in the urine in jaundice depends on the nature of the serum bile pigments and will be considered in the discussion following the previous contribution. The present communication will therefore be concerned only with those other end products of haemoglobin metabolism which appear in the faeces and which in certain circumstances may appear in the urine. After discussing briefly the possible pathways of haemoglobin breakdown, the three pigments—stercobilin (tetrahydromesobiliene), urobilin IXα (mesobiliene) and D-urobilin—and their respective precursors will be considered, together with the possible mechanism of their formation and possible pathways of their further breakdown. The enterogenous and hepatogenous theories of formation of the 'faecal urobilin' will be considered. The existence of three metabolic sources of bile pigment, the conversion of the bile pigments into substances not reacting as 'faecal urobilinogen' and the possible breakdown of haemoglobin via other pathways clearly invalidate 'faecal urobilinogen' excretion as an index of the rate of haemoglobin breakdown.

Methaemoglobin and Sulphaemoglobin. By Q. H. Gibson. (*Department of Physiology, University of Sheffield*)

Methaemoglobin (MetHb), in which the iron is present in the ferric state, is unable to combine reversibly with oxygen. It may be recognized in blood by its characteristic absorption spectrum, coupled with the changes in the spectrum following the addition of cyanide or hydrosulphite. It is found in the blood of normal persons in small quantities only, of the order of 0·5–2% of the total pigment, but in pathological conditions much larger amounts may be present. The small quantity normally present in blood appears to represent an equilibrium concentration at which the rates of formation and removal balance; in the methaemoglobinaemias this balance is disturbed. In toxic methaemoglobinaemia there is an increase in the rate of formation of MetHb; in some cases, at least, of idiopathic methaemoglobinaemia there is a decrease in the rate of removal.

The mechanism of removal of MetHb from the blood has been shown to be due to the action of intracorpuscular enzyme systems. In the treatment of methaemoglobinaemia their action may be speeded up by the catalytic action of certain dyestuffs, or supplemented by the direct reducing action of ascorbic acid.

The physiological effects of replacement of part of the haemoglobin by MetHb appear to be slighter than would be expected, since MetHb not only reduces the oxygen capacity of the blood, but has been shown to displace the oxygen dissociation curve, and to alter its shape in a way unfavourable for the efficiency of oxygen transport. In long-standing cases of idiopathic methaemoglobinaemia little if any disability has been present, even with amounts of MetHb as great as 40% of the total pigment.

The structure of sulphaemoglobin is not certainly known, and it has not yet been prepared in the pure condition. The iron is ferrous, but the compound does not combine reversibly with oxygen. Unlike methaemoglobin it does not occur in normal blood, nor does there seem to be any mechanism for its reconversion to haemoglobin; its rate of disappearance from the blood, indeed, affords a means of determining the life of the erythrocytes containing it. Its formation in the body is thought to depend on the production of hydrogen sulphide by intestinal bacteria, which, in the presence of some drugs such as phenacetin, acetanilide and the sulphonamides, attacks the haemoglobin. The concentration reached in the blood is seldom large; it is now known if its presence influences the oxygen dissociation curve.

The Chemical Pathology of the Carotenoids. By T. W. Goodwin. (*Department of Biochemistry, University of Liverpool*)

No function has yet been unequivocally demonstrated for carotenoids *per se* in mammals, but some carotenoids (β-carotene especially) are important because they can be converted into vitamin A as they traverse the gut wall. The two main conditions to be observed for normal metabolism of these pigments, are, therefore, (*a*) that they should be absorbed from the lumen, and (*b*) that the enzyme system carrying out the conversion should be intact and functional. The thyroid gland appears to be the main controller of the efficiency of absorption and also, possibly, of the conversion. The mechanism of conversion may be impaired in diabetes, although this has not yet been confirmed. As bile salts are necessary for the absorption of carotenoids, efficiency of absorption is reduced in jaundice and allied conditions.

In the past, numerous attempts have been made to correlate blood carotenoid levels with various pathological conditions; reasons will be given why, in the light of present knowledge, such investigations are likely to prove unprofitable.

An excessive intake of carotenoids in humans can lead to intense yellow pigmentation caused by the deposition of carotenoids in the hypodermis. This is generally only of cosmetic importance and usually quickly disappears following removal of carotenoids from the diet. There is only one recorded case in which carotenaemia appeared to be accentuated by the inability of the patient to convert carotene into vitamin A.

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

Metabolism of acetoin:

1. The formation and utilization of acetoin and butane-2:3-diol in the decerebrated cat. By J. DAWSON and R. P. HULLIN

2. Metabolic conversions of acetoin, pyruvate and acetate by rabbit-kidney tissue dispersions. By J. DAWSON and R. P. HULLIN

Rate of elimination of homologous globulins (including antibody) from the circulation. By J. H. HUMPHREY and A. S. MCFARLANE

Addendum—Assay of ^{14}C in the gas phase as carbon dioxide. By J. E. S. BRADLEY, R. C. HOLLOWAY and A. S. MCFARLANE

Fate of homologous antibody in the rabbit. By J. H. HUMPHREY and A. S. MCFARLANE

Some properties of creatine phosphokinase. By A. H. ENNOR and H. ROSENBERG

Studies in the biochemistry of micro-organisms. 92. The colouring matters of *Penicillium islandicum* Sopp. Part 4. Iridoskyrin, rubroskyrin and erythroskyrine. By B. H. HOWARD and H. RAISTRICK

The structure of the naturally occurring phosphoglycerides. 2. Evidence derived from a study of the action of phospholipase *C*. By C. LONG and M. F. MAGUIRE

Glyoxalase and methylglyoxal in thiamine-deficient rats. By H. M. SALEM

The lipoprotein particles in cow's milk. By R. K. MORTON

Studies on the labelling of brain phospholipids with radioactive phosphorus. By R. M. C. DAWSON

The incorporation of ^{65}Zn into avian eggs. By R. TUPPER, R. W. E. WATTS and A. WORMALL

The *in vitro* enzymic hydroxylation of steroid hormones. 1. Factors influencing the enzymic 11β-hydroxylation of 11-deoxycorticosterone. By A. C. BROWNIE and J. K. GRANT

Phosphoglyceric acid formation by carbon dioxide fixation in plant extracts. By E. W. FAGER

The ketonic carotenoid canthaxanthin isolated from a colour mutant of *Corynebacterium michiganense*. By S. SAPERSTEIN and M. P. STARR

Uridine compounds in glucuronic acid metabolism. 1. The formation of glucuronides in liver suspensions. By G. J. DUTTON and I. D. E. STOREY

Studies in immunochemistry. 14. The isolation and properties of substances of human origin possessing blood-group B specificity. By R. A. GIBBONS and W. T. J. MORGAN

Addendum—Physio-chemical examination of human blood-group B substances. By E. A. CASPARY

The branched-chain fatty acids of butterfat. 4. The isolation of (+)-12-methyltetradecanoic acid and of 13-methyltetradecanoic acid. By R. P. HANSEN, F. B. SHORLAND and N. JUNE COOKE

Rapid determination of salicylate in biological fluids. By P. TRINDER

The inhibition of trypsin and chymotrypsin by certain organic phosphorus esters. By B. A. KILBY and G. YOUATT

Studies on sulphatases. 7. A preliminary account of the glycosulphatase of *Littorina littorea*. By K. S. DODGSON and B. SPENCER

Adenosinetriphosphatase and hexokinase in the epithelium of the small intestine in normal and adrenalectomized rats. By E. LÜTHY and F. VERZÁR

The oligosaccharides produced by the action of yeast invertase preparations on sucrose. By J. S. D. BACON

The effect of temperature on the content and turnover of sodium and potassium in rabbit nerve. By H. MCLENNAN and E. J. HARRIS

The amino acid composition of some insect viruses and their characteristic inclusion-body proteins. By EUNICE F. WELLINGTON

Amino acid utilization in bacterial growth. 2. A study of threonine-isoleucine relationships in mutants of *Escherichia coli*. By H. AMOS and G. N. COHEN

Stoicheiometry of the citrulline phosphorylase reaction of *Streptococcus lactis*. By M. KORZENOVSKY and C. H. WERKMAN

A simple method for the preparation of horseradish peroxidase. By R. H. KENTEN and P. J. G. MANN

The effect of salicylate on liver glycogen in the rat. By M. J. H. SMITH

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University College, Gower Street, London, W.C.* 1, *on Saturday, 20th March,* 1954, *at* 10.45 *a.m. when the following papers were read:*

COMMUNICATIONS

The Effect of *p*-Nitrophenol on the Synthesis of Citric Acid in Fluoroacetate-blocked Mammary-gland Homogenates. By C. Terner. (*National Institute for Research in Dairying, University of Reading*)

The rate of synthesis of citric acid in the presence of fluoroacetate has been used as a measure of the rate of formation of 'active acetate' from pyruvate and acetate. The observation that the formation of citric acid in guinea-pig mammary-gland homogenates metabolizing fumarate and pyruvate in the presence of fluoroacetate is stimulated by *p*-nitrophenol (*p*NP, 2×10^{-4}M), has been reported in a previous communication (Terner, 1953). In this system, fluoride (0·01M) depressed the rates of respiration, pyruvate disappearance and citric acid formation to approximately the same extent and *p*NP reversed this inhibition. No stimulating effects corresponding to those of *p*NP were observed on addition of glucose and hexokinase. In the presence of the added phosphate acceptor system, *p*NP produced as much acceleration of respiration and citric acid synthesis as in its absence.

In the same fluoroacetate-blocked homogenate, fumarate plus acetate produced more citrate than fumarate alone, but considerably less than fumarate plus pyruvate. Since in previous experiments (Terner, 1953) in the absence of fluoroacetate the rate of citrate synthesis from acetate appeared to be at least as rapid as from pyruvate, it seems that the rate of activation of acetate in the fluoroacetate-blocked system is limited by the rate of phosphorylation accompanying the oxidation of fumarate. The addition of *p*NP, which abolished this phosphorylation, resulted in the almost complete inhibition of citric acid formation. Fluoride (0·005–0·01M) also strongly inhibited the formation of citrate from acetate and the effects of fluoride and *p*NP were additive. In accordance with these results it was found that the incorporation of ^{14}C-labelled acetate into the fatty acids of mammary-gland homogenates was strongly inhibited by *p*NP, fluoride or fluoroacetate.

Arsenate (0·005M) almost abolished the formation of citric acid from acetate, while in the presence of pyruvate much larger amounts of arsenate (0·02–0·04M) were required to produce a corresponding inhibition. Insulin did not affect the rate of citrate formation from either pyruvate or acetate.

Since the oxidation of pyruvate in the fluoroacetate-blocked system results in the generation of large amounts of phosphate-bond energy, the lack of available primary phosphate acceptors in the phosphorylating system would explain the restricted rate of oxidation of pyruvate and its release by nitrophenols which are believed to cause the breakdown of a phosphorylated intermediate (see Slater, 1953). The inhibitory effect of fluoride on the pyruvate-oxidase system (Liébecq & Peters, 1949) and its reversal by *p*NP may be interpreted in similar terms. To test this, α-oxoglutarate was added to homogenates metabolizing acetate or pyruvate in the presence of fumarate and fluoroacetate. The formation of citrate from pyruvate was inhibited by about 40%, and was accelerated by *p*NP to the same extent as in the absence of α-oxoglutarate. In the presence of acetate, however, α-oxoglutarate did not accelerate the synthesis of citric acid, but it retarded the inhibitory action of *p*NP.

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Gas-liquid Chromatography: The Separation, Micro-estimation and Identification of Hydrocarbons, Alkyl Halides, Alcohols, Aldehydes, Ketones, Esters and Ethers. By A. T. James and A. J. P. Martin. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Gas-liquid chromatography of volatile titratable substances was described by James & Martin (1952*a*), James, Martin & Howard-Smith (1952), James (1952) and James & Martin (1952*b*). Several general methods are available for the detection of vapours in gases, some of which have been described by Griffiths, James & Phillips (1952). Ray (1954), using the thermal conductivity method, has published an extensive series of separations using columns whose stationary liquid phase is dinonylphthalate. We have developed an apparatus for the detection of changes in gas density of the effluent

from the gas columns sensitive to 0·05 μg. of vapour/ml. of nitrogen (the mobile phase). This apparatus is believed to have advantages over any other yet described.

The essential advantage of the gas columns is that the detection of the substance to be analysed in the carrier gas is much easier than the corresponding problem of detecting substances in the moving solvent of ordinary chromatograms. The high speed of the method allows the use of long highly efficient columns which are capable of separating many isomers, e.g. six out of the eight isomers of *n*-hexane can be separated on a 4-foot column.

Different chemical groupings respond differently to changes in the column stationary phase and hence many deductions can be drawn as to the chemical nature of an unknown substance. The retention volume (the volume of nitrogen passing through the column before the centre of a zone emerges) of a particular substance in a defined stationary phase is a physical constant of the same nature as an R_F value and can in many cases provide unambiguous evidence of identity. The ease with which these retention volumes can be determined makes the method of particular value for the investigation of forces involved in solution.

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Interconversions Between 8-Azapurines. By R. E. F. Matthews and J. D. Smith. (*Agricultural Research Council Plant Virus Research Unit, Molteno Institute, Cambridge*)

The guanine analogue, 8-azaguanine, in which $C_{(8)}$ of the purine ring is replaced by nitrogen, is incorporated into the ribonucleic acid (RNA) of tobacco mosaic virus (TMV) (Matthews, 1953), *Bacterium coli*, *Staphylococcus aureus*, mouse sarcoma S37, mouse liver and spleen (Lasnitzki, Matthews & Smith, 1954) and germinating pea (*Pisum sativum*) seedlings.

We have now found that a number of similar compounds, related to 8-azaguanine (in which the corresponding carbon is replaced by nitrogen) are sufficiently similar to the natural compounds to be converted in biological systems to 8-azaguanine, and to be incorporated into various RNA's as 8-azaguanylic acid. These are 8-azaadenine (in TMV and *Bact. coli*), 8-azahypoxanthine (in *Bact. coli* but not in TMV), and 5-amino-1-*H*-1:2:3-triazole-4-carboxyamide the corresponding analogue of 5-aminoimidazole-4-carboxyamide (in TMV and *Bact. coli*). Certain related compounds were not incorporated. In all cases where incorporation was observed there was inhibition of the development of TMV or growth of *Bact. coli*. Conversely, where no incorporation could be detected no inhibition was found.

8-Azahypoxanthine was incorporated into *Bact. coli* RNA as 8-azaguanine to a greater extent than was 8-azaguanine itself. The simultaneous addition of hypoxanthine (0·0005M) together with 8-azahypoxanthine (0·005M) prevented both growth inhibition and incorporation in *Bact coli*. 8-Azahypoxanthine had no effect on development of TMV and was not incorporated as 8-azaguanine in the TMV RNA. Inhibition of growth of *Bact. coli* by 8-azahypoxanthine was not apparent until after 1·5–2 generation times when the growth rate progressively decreased.

5-Amino-1-*H*-1:2:3-triazole-4-carboxyamide inhibits development of TMV and growth of *Bact. coli* to about the same extent as does 8-azaguanine.

We have some evidence that 8-azaadenine is also incorporated into *Bact. coli* RNA as 8-azaadenylic acid.

None of these observations is inconsistent with the view (Lasnitzki, Matthews & Smith, 1954; Matthews, 1954) that the 8-azapurines cause growth inhibition through the production of biologically ineffective RNA.

We wish to thank the American Cyanamid Co. and Imperial Chemical Industries for gifts of compounds.

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The Effect of Salicylate on Liver Glycogen, Blood Glucose and the Passage of a Barium Meal in the Rat. By M. J. H. Smith. (*Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, S.E.* 5)

The intraperitoneal injection of salicylates in normal rats has been observed to cause a marked depletion of liver glycogen 4–7 hr. after the injection (Lutwak-Mann, 1942; Smith, Meade & Bornstein, 1952). The reduction was temporary, and partial replacement of the liver glycogen had occurred in 24 hr. The animals were given unrestricted access to food which was withdrawn immediately before the salicylate injection, and the fasting period of some rats may have been longer than others. This difficulty has been overcome by using rats trained to eat a single feed of 1 hr. duration per day (Barnes, 1953).

The injection of salicylate immediately after a meal caused an initial impairment of the deposition of liver glycogen and a fall in blood glucose, but after 24 hr. the levels of these substances were significantly higher than in control animals. One explanation of these effects was a delayed absorption of food from the gut, and the effect of salicylate on the passage of a barium meal in the rat has therefore been investigated (Smith & Irving, unpublished). The intraperitoneal or intravenous injection of salicylate caused a considerable delay in gastric emptying. In the normal rat the stomach is evacuated of barium in 2 hr., but in the salicylate-treated animal the barium was retained in the stomach for 12 hr. and a considerable proportion remained after 15 hr.

It is concluded that the effects of salicylate on liver glycogen and blood glucose described above are due to this prolonged time of gastric emptying although such a mechanism cannot explain other actions of salicylate on carbohydrate metabolism (Ingle, 1950; Smith *et al.* 1952).

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Factors Involved in the Formation of the β-Anomaly During the Electrophoresis of Human Serum Proteins. By E. M. Crook, M. K. El-Marsafy and E. M. Shooter. (*Department of Biochemistry, University College, London*)

The β-anomaly, so-called, is well known to appear in the descending limb but not in the ascending limb during the electrophoresis of normal human serum proteins (Longsworth, Shedlovsky & MacInnes, 1939). Explanations of this phenomenon involving reflexions due to turbidities or to interactions among the serum proteins (Abramson, Moyer & Gorin, 1942) are scarcely tenable with our present knowledge of electrophoretic systems. Experiments with a series of increasing currents now show that the β-anomaly depends not so much upon the separation of the components as upon their rate of separation. The more rapidly a given state of separation is reached, the more pronounced is the β-anomaly. It is not, however, due to heating by the current, since a.c. currents of the same magnitude cause no β-anomaly.

The production of schlieren diagrams similar to the β-anomaly by artificial inverted density gradients strongly suggests that it is due to convection. Longsworth (1945) also suggested this explanation. The a.c. experiments rule out thermal convection, and gravitational instability in the β-region remains as the most plausible cause of the convection. The following general equation relates the density change across any boundary to the composition and the properties of the adjacent phases:

$$\Delta D = \Sigma C_j \left(1 - \frac{\phi_j}{\phi_{X_i}}\right),$$

where the C's are concentrations (g./unit vol.) and the ϕ's are apparent partial specific volumes. j indicates all components, including the solvent, present on both sides of the boundary and X_i the boundary-forming component. Detailed qualitative application of this equation shows: (1) that all boundaries on the descending side are less stable than those on the ascending side, (2) that as ϕ_{X_i} is increased the boundary becomes less stable and at a certain value a negative density gradient may be produced. In view of (1), this will appear first on the descending side. A corollary will be that the more rapidly the separation occurs the larger will be the negative gradient and the more pronounced the convection, and vice versa.

The serum constituent having properties most likely to give rise to the β-anomaly is the β_1-lipoprotein of Oncley (1950) ('*X*-protein' of Pedersen, 1945). Taken in conjunction with the known properties of the β_1-lipoprotein (Oncley & Gurd, 1953), the following phenomena are in accord with this

suggestion: (1) disappearance of the β-anomaly in infected sera; (2) shift of the β-anomaly to the α_2-globulins in many aged sera; (3) dried sera show either no anomaly or a pronounced turbidity in the same region.

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Association of Carotene with Serum Proteins. By E. M. Crook and M. K. El-Marsafy. (*Department of Biochemistry, University College, London*)

The carotene present in human serum has been reported to be associated with albumin (Dzialoszynski, Mystkowski & Stewart, 1945), with globulin (Bennhold, 1938; Pett & LePage, 1940), with β-globulin (Troitzky, 1950), and with β_1-lipoprotein (Oncley, 1950). Because of this lack of agreement, a detailed investigation was undertaken, using the electrophoretic fractionation technique previously described (Crook & El-Marsafy, 1952), the carotene being estimated by the microspectrophotometric technique of Bessey, Lowry, Brock & Lopez (1946). Both the integral and derivative curves of the carotene concentration (expressed in μg. β-carotene %) were plotted as functions of the height in the U-tube.

Comparison of these curves with the conventional schlieren patterns shows that:

(i) No carotene is bound by the γ-globulin fraction.

(ii) The carotene is distributed among the other fractions approximately in the following proportions: β-globulin, 50%; albumin, 20%; α_2-globulin 10%; α_1-globulin, 7%.

It was also possible to calculate the order of magnitude of the carotene binding capacity of the various serum components (expressed as μg. β-carotene per gram protein component). These calculations are complicated by the overlap of certain of the boundaries and by the boundary anomalies. However, good agreement is found between the values for the fraction of the total carotene bound by the individual proteins using either the integral or the derivative curves. Apparent differences in the carotene binding capacity of certain components in the two limbs of the U-tube illustrate, and may be explained quantitatively, by boundary anomalies. The binding capacity of the components is in the following order:

$$\beta\text{-globulin} > \alpha_2\text{-globulin} > \alpha_1\text{-globulin} > \text{albumin} > \gamma\text{-globulin}.$$

There is tentative evidence suggesting the existence of two components in β-globulin with varying amounts of carotene bound to each. It is suggested that the component binding most carotene may be identical with the β_1-lipoprotein of Oncley (1950), while the one binding less carotene may be similar to one or more of the other β components defined by Edsall (1950).

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Colorimetric Determination of Dipeptide Hydrolysis. By E. M. Crook and B. R. Rabin. (*Department of Biochemistry, University College, London*)

Methods for the determination of dipeptide hydrolysis based on the ninhydrin colour inherently lack precision because of the large blank from the dipeptide itself. The same is true of titration procedures which have the blank further increased by any buffer present in solution. Gasometric procedures are so slow as to limit severely the number of estimations that can be performed. Many simple dipeptides give highly coloured oxygenated cobalt complexes (Gilbert, Otey & Price, 1951), and this reaction has now been rendered quantitative by careful control of the conditions and the use of hydrogen peroxide as oxygenation reagent.

Cobalt phosphate suspension is prepared by mixing thoroughly 2 vol. of 0·2M-Na_3PO_4 with 3 vol. of 0·2M-$CoCl_2$ and adding 10 vol. of 0·1M-$Na_2B_4O_7$, 10H_2O (final pH = 9·1).

Approximately 80 μmoles of dipeptide in 4 ml. of solution deproteinized with perchloric acid (Grauer, Mandl, Strauss & Neuberg, 1950) is made alkaline to thymolphthalein by the addition of NaOH. 10 ml. of cobalt phosphate suspension is added and the mixture placed in a boiling water bath. At the end of three successive 10 min. intervals, 0·5 ml. of 2 vol. H_2O_2 is added dropwise with constant shaking, and heating is continued 30 min. after the third addition. The solution is cooled, made up to 25 ml. with water, filtered through a Whatman no. 2 paper and read in the colorimeter using an Ilford 624 (green) filter.

The complexes have absorption maxima at 520 mμ. The intensity, in the absence of amino acids, is linear with concentration of dipeptide in the range 0–80 μmoles. Most amino acids, although producing almost no colour, destroy the linearity, presumably owing to the formation of mixed complexes. However, calibration curves of absorption/percentage hydrolysis of dipeptide are accurately reproducible, and the method gives results reproducible within the precision of the colorimeter.

The method has been tested on glycylglycine, DL-alanylglycine, DL-leucylglycine, glycyl-DL-alanine, DL-alanyl-DL-alanine, glycyl-DL-phenylalanine, glycyl-DL-leucine, glycyl-L-tryptophan, glycyl-L-tyrosine. The latter two dipeptides give atypical colours. Simple amino acids, glutamic and aspartic acids, serine, proline, phenylalanine, arginine, asparagine and ammonium salts give almost no colour. Tryptophan, tyrosine, histidine and cysteine give substantial and atypical colours. Excessive amounts of phosphate interfere and the method cannot be used in the presence of aminotrishydroxymethylmethane (Tris) buffer.

One of us (B.R.R.) is indebted to the Medical Research Council for a personal grant.

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Extending the Range of Colorimetric Measurement. By P. A. T. Swoboda* and E. M. Crook.
(*Department of Biochemistry, University College, London*)

To determine accurately inorganic phosphate covering a wide range of concentrations the technique of differential colorimetry has been employed. This technique (Körtum, 1937; Ringbom, 1939) has previously been used chiefly to obtain greater precision in measurement, e.g. Hiskey (1949).

The principle is to compare the sample solution (extinction E_x) with a standard solution of the same substance by setting the colorimeter at zero with the standard instead of the blank. Assuming Beer's Law to hold, the concentrations (c) are given by

$$c = kE = k \log \frac{I_0}{I},$$

and the difference in concentration

$$c_x - c_{st} = k\left(\log \frac{I_0}{I_x} - \log \frac{I_0}{I_{st}}\right) = k \log \frac{I_{st}}{I_x}.$$

If now the sensitivity of the colorimeter is adjusted so that a full-scale deflexion ($\theta = 100$) is obtained with the standard solution, then

$$c_x - c_{st} = k \log 100/\theta_x,$$

since θ is proportional to I. The factor $\log 100/\theta_x$ is the usual scale marked on photoelectric instruments, and so the difference in concentration is given directly by the scale reading, the proportionality constant k being the same as in the absolute determination of extinction (where $c_{st} = 0$).

This technique has been tested on several types of instruments with the inorganic phosphate method of Allen (1940). Extinctions up to 1·6 have been measured in four ranges using standards of 0, 100, 200 and 300 μg. phosphorus per 25 ml., having extinctions of approximately 0, 0·4, 0·8 and 1·2 respectively. The precision with which the unknown concentration is determined increases with increasing standard concentration on the differential ranges and is maintained even for low-scale readings.

The colorimeter sensitivity may be increased by increasing the light intensity or by control of the galvanometer sensitivity. There is a possibility that these may alter the proportionality constant k by introducing non-linearity of response, and this should be checked when any particular instrument is used for this technique.

An additional extension of the sensitivity range can be made by using a less dense colour filter. An Ilford 204 (red) filter was used here, instead of

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the Ilford Spectrum Red 608 without producing any deviations from Beer's law.

It is suggested that this technique be applied to other colorimetric estimations, increasing their range and precision and avoiding the tedium of diluting concentrated solutions.

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The Transformation of Purines into Pteridines. By A. Albert. (*Department of Medical Chemistry, The Australian National University, 183 Euston Road, London*)

When 2-hydroxypurine (Albert & Brown, 1954) is incubated with glyoxal at 37°, it is converted into 2-hydroxypteridine in 84% yield. The best solvent was found to be N-H_2SO_4 (24 hr.), but even in water 16% of the pteridine had formed in a week. Under more severe conditions, a violet precipitate was formed by the destructive action of acids upon 2-hydroxypteridine (Albert, Brown & Cheeseman, 1951).

Similar transformations of other purines into pteridines have been achieved (e.g. guanine into xanthopterin), and conditions for obtaining comparable high yields are being sought.

If this reaction between purines and 1:2-dicarbonyl substances is found to be a general one, light may be shed on the biosynthesis of pteridines (which are themselves necessary catalysts for the formation of purines). At a higher level of organization, pteroylglutamic acid has been shown to catalyse mammalian mitosis (Jacobson, 1952), and xanthopterin vastly increases the mitotic rate in mammalian kidneys which then grow to many times their normal size (Haddow, 1947). Hence it is interesting to speculate whether localized excessive growth could be caused by a local accumulation of 1:2-dicarbonyl substances which could convert the abundant natural purines into these or similar pteridine catalysts.

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The Effect of Methylandrostenediol on the Growth Rate and Composition of the Rat. By A. Korner. (*Department of Biochemistry, University of Cambridge*)

Methylandrostenediol (17-α-methylandrost-5-ene-3β:17β-diol) is only weakly androgenic but it induces protein anabolism (Gordon, Eisenberg, Moon & Sakamoto, 1951). Limit-fed female rats treated each day with 0·3 mg. of methylandrostenediol by subcutaneous injection in propylene glycol gained more weight than control animals consuming the same ration. These differences in weight gain were highly significant ($P < 0{\cdot}001$). The effect on weight obtained varied with the age of the animals, and the optimum growth response was found with rats which had just stopped rapid growth ($3\frac{1}{2}$–4 months old).

The water, fat and protein content of the total minced tissues of the rats were determined, and it was shown that the treated animals had a higher protein and water content and a lower fat content than their controls. These differences in composition were statistically significant ($P < 0{\cdot}01$). It was calculated that the material added had a composition similar to that of muscle.

It was shown that there was a significant decrease in the weight and protein content of the ovaries and uterus of the treated animals, and therefore the new protein must have been laid down in non-sexual tissue.

These results are similar to the changes in composition observed when rats are treated with anterior pituitary extract (Young, 1945).

I am indebted to Mr C. W. S. Taylor of CIBA Laboratories for a gift of the methylandrostenediol used.

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The Contribution of Dietary Stearic Acid to Milk Fat. By W. G. DUNCOMBE and R. F. GLASCOCK. (*National Institute for Research in Dairying, University of Reading*)

Isotope experiments have shown that acetate is incorporated into all the fatty acids of ruminant milk (for review see Folley, 1952). Its contribution to stearic and oleic acids is small, however, and their origin remains uncertain. Earlier work using the arterio-venous technique (e.g. Graham, Jones & Kay, 1936) showed that the mammary gland absorbs appreciable quantities of blood neutral fat which might therefore be the chief source of long-chain acids of milk fat.

Stearic acid, labelled with tritium predominantly at the $C_{(9)}$ and $C_{(10)}$ positions by the tritiation of oleic acid (Glascock, unpublished), has been fed to a lactating goat. A blood sample was taken after 7 hr. and milk collected daily. 17% of the ingested activity was secreted in the milk lipids in the first 24 hr., and 25% in the first 5 days. Small but measurable activity was still being secreted after 16 days.

By taking the average daily ingestion of glyceride long-chain fatty acids as 150 g. and assuming that, as shown by Borgström (1952) for the rat, randomization of free and glyceride fatty acids occurs in the gut, it is calculated that approximately 70% of the long-chain fatty acids of milk are derived from dietary fat (presumably transported as blood lipids).

Fractionation of the first day's milk lipids showed that saturated and unsaturated non-volatile acids from glycerides and total non-volatile acids from phospholipids had about the same specific activity as acids from blood glycerides.

Smaller but significant activity was also found in the blood-plasma volatile acids and in the following milk constituents: steam volatile glyceride and phospholipid fatty acids, glyceride glycerol, lactose, cholesterol and water. This shows that dietary stearic acid not only contributes to long-chain fatty acids but may also contribute to other milk constituents.

Since the oleic acid used for preparing the labelled stearic acid may have contained small quantities of other unsaturated acids the product may not, therefore, have consisted exclusively of [9:10-^{3}H]stearic acid. For this reason pure crystalline elaidic acid has been used for a second preparation, thus ensuring that the tritium is located only in the $C_{(9)}$ and $C_{(10)}$ of stearic acid. Further experiments are in progress using this material.

Thanks are due to Dr S. J. Folley, F.R.S., for making available the experimental animal; to Dr D. S. Flux for administering the labelled material and for taking blood samples; and to Mr B. W. E. Peaple for tritium estimations.

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The Metabolism of Quinoline. By J. N. SMITH and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

In intact animals, quinoline is hydroxylated in the 3-, 6- and 8-positions (Novack & Brodie, 1950; Scheunemann, 1923); rabbit-liver extracts oxidize it in the 2-position (Knox, 1946). In dogs and hens it is methylated in the 1-position (Tamura, 1924). A re-examination of the problem shows that the metabolism of quinoline is very complex. By isolation we find that quinoline (0·2 g./kg.) yields in rabbits 3-hydroxy-, 5:6-dihydroxy- and 2:6-dihydroxy-quinoline. Paper chromatography of the urine suggests the presence of 6-, 7- and 8-hydroxyquinolines, but this is unsupported as yet by isolation. The main component of the ethereal sulphate fraction (8% of the dose; Smith, 1953) of urine from quinoline-treated rabbits appears to be *6-hydroxyquinolyl-5-sulphuric acid* (1·5% of dose by isolation; 3% by colorimetric estimation) which yields 5:6-dihydroxyquinoline (picrate, m.p. 205°) on acid hydrolysis. We have synthesized the above ethereal sulphate by an Elbs persulphate oxidation of 6-hydroxyquinoline. 5:6-Dihydroxyquinoline appears to be the main component of the ethereal sulphate fraction, but small amounts of other hydroxyquinolines can be detected by paper chromatography.

The glucuronide fraction (40% of the dose) on acid hydrolysis yielded mainly a black unidentified solid. So far only small amounts of 3-hydroxyquinoline (picrate, m.p. 250°) and 6-hydroxy-2-quinolone (6-acetyl derivative, m.p. 225°) have been isolated from this fraction.

The urine also contains an acid-labile compound which yields quinoline on heating with acid (cf. Tamura, 1924). The quinoline formed in this

way was steam-distilled and estimated spectrophotometrically, and corresponded to 10% of the dose.

Thus it appears that positions 1, 2, 3, 5, 6, 7 and 8 of quinoline may undergo metabolic change. Scheunemann's (1923) identification of 6-hydroxy-4-quinolone as a quinoline metabolite is probably erroneous.

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The Metabolism of Dichlorobenzenes. By W. M. Azouz, D. V. Parke and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

Smith, Spencer & Williams (1950) showed that the major metabolites of monochlorobenzene in rabbits were *p*-chlorophenylmercapturic acid and 4-chlorocatechol conjugates. We have now found that mercapturic acids and catechols are not the major metabolites of dichlorobenzenes, the *o*- and *p*-isomers of which are used as pesticides. When fed to rabbits the dichlorobenzenes (0·5 g./kg.) are slowly metabolized, the excretion of metabolites occurring during 3–6 days after dosing. A considerable part of the dose is excreted as glucuronides and ethereal sulphates. Mercapturic acids and catechols are minor metabolites of *o*- and *m*-dichlorobenzenes and do not appear to be formed after the *p*-isomer (cf. Baumann, 1883; Callow & Hele, 1926). With the *o*-isomer, the metabolites are glucuronides 48%, sulphates 21%, mercapturic acid 5% and catechols 4% of the dose. The major metabolites are conjugates of 3:4- and 2:3-dichlorophenols which are excreted roughly in the ratio 2:1. The catechol fraction is mainly 4:5-dichlorocatechol with traces of 3:4-dichlorocatechol. The mercapturic acid is probably 3:4-dichlorophenylmercapturic acid. With the *m*-isomer (glucuronides 31%, sulphates 11%, mercapturic acid 9%, and catechols 4%) the major product is 2:4-dichlorophenol with traces of 3:5- but no 2:6-dichlorophenol. The mercapturic acid is 2:4-dichlorophenylmercapturic acid and the catechol, 3:5-dichlorocatechol. *p*-Dichlorobenzene (glucuronides 37%, sulphates 27% and mercapturic acid and catechols 0%) yields 2:5-dichlorophenol and a quinol which is probably 2:5-dichloroquinol.

These results will be discussed.

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Paper Chromatography of Vitamin D and other Sterols. By E. Kodicek and D. R. Ashby. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

The reversed-phase partition chromatography of sterols on filter paper impregnated with 'Quilon' (stearato chromic chloride) has been reported recently (Kritchevsky & Calvin, 1950; Kritchevsky & Kirk, 1952). Thus Davis, McMahon & Kalnitsky (1952) were able to separate ergocalciferol and cholecalciferol from 7-dehydrocholesterol, ergosterol, cholesterol and sitosterol. We have confirmed these results, but found that lumisterol and suprasterol II have the same R_F values as vitamin D.

We have used filter paper impregnated with liquid paraffin B.P. (Eggitt & Ward, 1953). When Whatman no. 2 paper was coated by dipping in 5% (v/v) liquid paraffin dissolved in light petroleum a number of solvent systems were found which separated vitamin D from other sterols. More than 100 solvent combinations were examined and the following were found to be the most suitable ones: (A) ethyleneglycol monoethyl ether 35/*n*-propanol 10/methanol 30/water 25; (B) *n*-propanol 15/methanol 82/water 3; (C) methanol 95%. Ascending chromatograms of the sterols (20 μg.) were run for 16–17 hr., the papers were dried at room temperature, warmed on a hot plate (50–70°) and immediately sprayed with a 24% solution of $SbCl_3$ in chloroform. The papers were then warmed until the colour of the sterols appeared. In ultraviolet light (365 mμ.), as little as 1 μg. of vitamin D can be detected by its fluorescence. It was found useful to examine the chromatograms, before spraying, with ultraviolet light (256 mμ.), when certain sterols appear as light-absorbent spots. The mean R_F values of the following substances were determined (for solvents A, B and C, respectively): ergocalciferol (0·44, 0·66, 0·69), cholecalciferol (0·46, 0·68, 0·68), 7-dehydrocholesterol (0·39, 0·57, 0·56), ergosterol (0·0, 0·56, 0·0), lumisterol (0·27; 0·59, 0·52), supra-

sterol II (0·36; 0·52, 0·57), cholesterol (0·29, 0·49, 0·50), sitosterol (0·0, 0·51, 0·42), zymosterol (0·41, 0·61, 0·48), α-tocopherol (0·15, 0·58, —), γ-tocopherol (0·26, 0·72, —), δ-tocopherol (0·45, 0·77, —), α-, β- and γ-carotene (0, 0, 0). Vitamin A alcohol (20 μg.) gave two spots R_F 0·93, 0·98, in solvent A. It can thus be distinguished when present in equimolar amounts with vitamin D, provided that no other breakdown products are obtained. Tachysterol, apart from the main spot, R_F 0·37, 0·61 and 0·58 in solvents A, B and C, showed two other spots, R_F 0, 0·98, in all three solvent systems which on elution gave a different absorption spectrum from tachysterol. When large amounts of vitamin D (100–1000 μg.) were chromatographed in either of the solvents, a second spot (R_F 0·98) could be seen which on elution had an absorption maximum at 248 mμ.

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The Estimation of Vitamin D by Paper Chromatography. By E. Kodicek and D. R. Ashby.
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The paper-chromatographic technique described in the previous communication (Kodicek & Ashby, 1954) has been applied to the quantitative estimation of vitamin D. The non-saponifiable matter (20–30 mg.) of some biological substances was chromatographed on a column (12 cm.) of magnesium oxide: kieselguhr (50:50) with 100 ml. light petroleum (b.p. 40–60°) containing 0·5 % (v/v) acetone. After evaporation of the solvent *in vacuo*, the residuum was taken up in methanol and precipitated with digitonin. After 17 hr., the suspension was extracted four times with equal volumes of light petroleum. After evaporation of the extract *in vacuo*, the residuum was taken up in 0·2 ml. ethanol. The extract (20 μl.) was applied on strips of Whatman no. 2 filter papers (1·5 cm. wide) and developed with the solvent systems described (Kodicek & Ashby, 1954). In each experiment, a standard vitamin solution was added to the extract and the recovery was used for the calculation of the vitamin content of the extract. After 16–17 hr., the strips were examined under ultraviolet light (256 mμ.), and the dark absorbent region, containing the vitamin, was cut out and eluted with 5 ml. methanol at room temperature. Blank strips of filter paper were similarly eluted and the solutions were read on a Unicam spectrophotometer at a wavelength of 265 mμ. The mean percentage recovery of ergocaliciferol (50 experiments) applied on paper strips and chromatographed was 92 % ± 12·1 (standard deviation). The recovery of ergocalciferol added to the investigated material (26 samples) was 88 % ± 10·6 (standard deviation). The vitamin D content of 1 g. samples of arachis oil, to which 120, 240 and 500 μg. ergocalciferol had been added, was found to be 126, 220 and 431 μg., respectively. Ostelin, Glaxo Ltd., containing 5000 i.u. (125 μg.) of vitamin D per ml., had an estimated potency of 117 μg./ml. Faeces, collected for 24 hr., from three groups of rachitic rats, which were given a single massive dose of 1 mg. ergocalciferol *per os*, were also assayed. The biological potency of these samples had been previously determined (Cruickshank, Kodicek & Armitage, 1953), i.u./rat: group *a*, 6460; group *b*, 3070; group *c*, 3670. The corresponding values, determined chemically, were: 148 μg. (5920 i.u.), 134 μg. (5360 i.u.), 103 μg. (4010 i.u.). It appears that the vitamin D content of biological material can be determined by the chromatographic procedure, provided that more than 25 μg. vitamin D per g. is present and vitamin A is absent. Large amounts of vitamin A interfere with the estimation, and work is in progress to eliminate this interference.

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Coenzyme *A* Dependent Reactions in a Soluble Enzyme Preparation from Rat Mammary Gland.

By G. Popják* and A. Tietz.† (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

The preparation of a soluble enzyme system from rat mammary gland, which synthesizes short- and long-chain fatty acids, has been reported previously (Popják & Tietz, 1953, 1954*a*, *b*). This soluble enzyme preparation requires only the addition of ATP (0·01 M) to effect a synthesis of fatty acids from acetate. The addition of α-oxoglutarate (0·02 M) to the system produced a 3–8-fold increase of the fatty acid synthesis, which was augmented further about five times by the addition of malonate (0·05 M).

Evidence has now been obtained to show the formation of acetyl coenzyme *A*, or generally acyl coenzyme *A* in our preparation, and also of the dependence of the fatty acid synthesis on certain co-factors.

Assay of the mammary gland enzyme preparation (MGE) for coenzyme *A*, carried out on boiled enzyme by the method of Kaplan & Lipmann (1948), showed that the MGE contains 20–25 units per ml. When incubated in the presence of acetate (0·05 M), ATP (5–10 μmoles) and of 0·5 M salt-free hydroxylamine the formation of hydroxamic acid derivatives could be shown (Lipmann & Tuttle, 1945). The formation of 'active acetate' was further proved by coupling the MGE with the pigeon-liver 'arylamine acceptor' enzyme system (A 60) of Chou & Lipmann (1952). While neither the MGE nor the A 60 alone could acylate sulphanilamide, 80–100 μg. were acylated by a mixture of 0·1 ml. MGE and 1·0 ml. of A 60, when incubated in the presence of 1 μmole sulphanilamide, 5 μmoles ATP and 14 units of coenzyme *A*. No further acylation was obtained by the addition of the substrates: acetate (0·02 M), α-oxoglutarate (0·02 M) or of malonate (0·05 M) either alone or combined.

The dependence of fatty acid synthesis on coenzyme *A* and on DPN was shown by experiments in which these cofactors were removed from the MGE by treatment with Dowex 1 ion-exchange resin, mesh size 200–400 (Chantrenne & Lipmann, 1950). The Dowex treatment resulted in an almost complete removal of coenzyme *A* and a loss of the fatty acid synthesis. Full reactivation was obtained by the addition of boiled MGE and ATP (0·01 M) or of coenzyme *A* (40 units), DPN (1·5 mg.) and ATP (0·01 M). The Dowex treatment resulted also in an almost complete loss of the hydroxamic acid reaction. Full reactivation was obtained by the addition of 14–20 units of coenzyme *A* and 5–10 μmoles of ATP. The coenzymes used so far have been crude preparations and the work is being extended with pure specimens.

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† Holder of a Glaxo Laboratories and the Friends of the Hebrew University Scholarship.

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The Use of Sodium Borohydride in the Determination of 17-Hydroxylated Corticosteroids.

By Joan I. Appleby, G. Gibson, J. K. Norymberski and R. D. Stubbs. (*Rheumatism Research Unit, Nether Edge Hospital, Sheffield* 11)

Sodium borohydride is known to effect the smooth conversion of oxosteroids into hydroxysteroids under mild conditions (for references see Brown & Mead, 1953). It is now found that, when combined with the bismuthate technique (Brooks & Norymberski, 1953; Norymberski, Stubbs & West, 1953), the reduction of oxosteroids with sodium borohydride offers new means of selectively estimating (i) all 17-hydroxylated corticosteroids ('total 17-OH C.S.'), and (ii) 17-hydroxy-20-oxosteroids unsubstituted at $C_{(21)}$.

The determination of urinary 'total 17-OH C.S.' is performed by treating urine with sodium borohydride (5 mg./ml.) overnight at room temperature and subsequently with sodium bismuthate under conditions previously described (Norymberski *et al.* 1953). The 17-oxosteroids thus formed and subsequently determined are derived exclusively from 17-hydroxylated steroids including 17-hydroxy-20-oxosteroids unsubstituted at $C_{(21)}$; all other ketones originally present in the urine are reduced to alcohols and cannot contribute to the Zimmermann colour. In these respects the advantage of the method over the estimation of 17-ketogenic steroids is evident.

The selective determination of 17-hydroxy-20-oxosteroids unsubstituted at $C_{(21)}$ in the presence of other corticosteroids is carried out in three steps:

(i) oxidation with sodium bismuthate, (ii) reduction with sodium borohydride (in 80% *tert.*-butanol overnight at room temperature), and (iii) oxidation with sodium bismuthate. In step (i) all 17-hydroxylated corticosteroids other than those to be estimated are converted to 17-oxosteroids (Brooks & Norymberski, 1953) which, together with any other ketones originally present, are reduced to alcohols in step (ii). Consequently, the only 17-oxosteroids present in the mixture of products obtained from step (iii) are those derived from 17-hydroxy-20-oxosteroids unsubstituted at $C_{(21)}$. The application of this procedure to urines is being investigated.

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Phospholipid Metabolism in Brain and Liver. By R. M. C. Dawson.* (*Department of Biochemistry, University of Oxford*)

A method has been devised which enables measurements to be made of the ^{32}P labelling of the individual kephalins and lecithin in a small sample of tissue (150–400 mg. fresh tissue). The lipids extracted from the tissue are mildly hydrolysed with alkali, and after the removal of alkali and fatty acids identifiable phosphorus-containing hydrolysis fragments of the phospholipids are completely resolved by two-dimensional paper chromatography.

The method has been used to study the deposition of ^{32}P in the lipids of a guinea-pig brain dispersion which had been incubated with labelled phosphate. The results confirmed that in such dispersions there was a negligible phosphorus replacement in phosphatidylethanolamine, phosphatidylserine or phosphatidylcholine. At the same time there was an active synthesis of both diphosphoinositide and a lipid which showed properties identical with those of phosphatidic acid.

In further studies the specific radioactivities of rat-liver phospholipids were measured at various time intervals after the animals had been injected with labelled phosphate. The maximum specific radioactivities of liver phosphatidylethanolamine, phosphatidylcholine and 'diphosphoinositide' were all reached at about 9–12 hr. after injection, but the maximum specific radioactivity of phosphatidylethanolamine was approximately 50% greater than that of phosphatidylcholine and 'diphosphoinositide'. From the water-soluble fraction of the same livers free glycerophosphorylcholine and glycerylphosphorylethanolamine were isolated by two-dimensional paper chromatography with phenol/NH_3 and propanol/NH_3 solvents after cations had been removed with a cation-exchange resin. This enabled specific radioactivity/time curves to be constructed for these compounds. When these curves were compared with similar ones constructed for liver phosphatidylcholine and phosphatidylethanolamine, it became apparent that glycerylphosphorylcholine could not be the immediate P precursor of phosphatidylcholine, nor glycerylphosphorylethanolamine the precursor of phosphatidylethanolamine. On the other hand liver phosphatidylcholine fulfilled the correct radioactive criteria for being the precursor of glycerylphosphorylcholine, while similarly liver phosphatidylethanolamine fulfilled the conditions for being the precursor of glycerylphosphorylethanolamine. These results point to the presence of a lecithinase *B* enzyme in liver, and also a similarly acting kephalinase and indicate one of the pathways of phospholipid catabolism in the liver *in vivo*.

* Betty Brookes Fellow.

Phosphatase Activities of Various Regions of Rabbit Bone. By H. R. Perkins. (*Institute of Orthopaedics, Brockley Hill, Stanmore, Middlesex*)

Adenosine diphosphoric acid (ADP) and adenosine triphosphoric acid (ATP) are known to form complexes with calcium (Di Stefano & Neuman, 1953) and ATP is known to be present in pre-osseous cartilage (Albaum, Hirshfeld & Sobel, 1952). Similarly, pyrophosphate and tripolyphosphate also form stable calcium complexes (e.g. Gosselin, Tidball, Megirian, Maynard, Downs & Hodge, 1953). Di Stefano & Neuman (1953) claimed that the inhibitory action of ATP on calcification of rachitic bone slices *in vitro* was accounted for by calcium-ATP complex formation, and by the slow liberation of inorganic phosphorus from ATP by that tissue. It therefore seemed of interest to examine

quantitatively the phosphatase activity of normal bone against the aforementioned substrates, together with β-glycerophosphate and adenosine-monophosphate (AMP), at physiological pH. Litter-mate rabbits about 2 months old were employed, the tibiae and femora being dissected out and scraped free of adhering tissue. The epiphyseal plate, metaphyseal and diaphyseal cortical regions were dissected out and ground with chloroform-saturated water. Samples of fresh and boiled homogenate were incubated with the various substrates at 37° and pH 7·4 for 15 min., and the inorganic phosphate liberated was then determined. (With pyrophosphate and tripolyphosphate it was necessary to precipitate any remaining substrate with Mn at pH 5·0 before phosphate determination.) It was found that the enzyme activity of the cortex was low throughout, although even in the boiled samples substrates forming stable Ca complexes liberated considerable amounts of inorganic phosphate from the tissue itself. The highest activity observed was that of metaphysis with ATP as substrate. The relative activity of metaphysis to epiphyseal plate varied with the substrate employed. Thus with AMP and glycerophosphate the activities were if anything higher in the epiphyseal plate region, but with ADP, ATP, pyrophosphate and tripolyphosphate the reverse was true. The agreement between the activities against pyrophosphate and tripolyphosphate was in all cases so close as to suggest that the same enzyme was probably involved.

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DEMONSTRATIONS

Fractionating Cells and other Equipment for the Tiselius Electrophoresis Apparatus Constructed from Perspex. By E. M. Crook, M. K. El-Marsafy, E. M. Shooter and F. B. Ward. (*Department of Biochemistry, University College, London*)

Addition of two simple 'fractionation plates' to the conventional Tiselius apparatus (1937), enables fractions to be obtained without serious mixing or dilution with buffer, from all levels in both limbs of the U-tube without removing it from the bath, or dismantling. The fractionation occurs under the same conditions as obtain during an analytical run, and the fractions are accurately defined with respect to the schlieren diagram. A 'special conductivity plate' has also been designed. This allows simultaneous fractionation, and microconductimetric measurements on the contents of the U-tube.

These modifications have proved convenient even for everyday use of the apparatus since they simplify assembly and filling and have therefore been incorporated as an integral part. The modified cell assembly, consisting of three sections only, is fabricated entirely from Perspex. The schlieren diagrams obtained with Perspex centre sections are of the same quality as those obtained with the available glass sections. The dimensions of the apparatus, which is easily made with very moderate workshop facilities, are readily altered to suit the requirements of particular experiments. A preliminary description of this apparatus was given to the Second International Congress of Biochemistry (1952).

On view will be several modified cell assemblies in Perspex.

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Differential Colorimetry Applied to Extend the Range of Phosphate Estimation. By P. A. T. Swoboda. (*Department of Biochemistry, University College, London*)

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

The biosynthesis of penicillin:

1. The incorporation of some amino acids into penicillin. By H. R. V. ARNSTEIN and P. T. GRANT
2. The incorporation of cystine into penicillin. By H. R. V. ARNSTEIN and P. T. GRANT

The tryptophanase-tryptophan reaction. The nature of the enzyme-coenzyme-substrate complex. By H. GOODER and F. C. HAPPOLD

A note on the action of fluoroacetate. By J. D. JUDAH and K. R. REES

Studies in carotenogenesis. 11. Carotenoid synthesis in the alga *Haematococcus pluvialis*. By T. W. GOODWIN and MALINI JAMIKORN

The effect of 3 : 5-dinitro-*ortho*-cresol on phosphocreatine and the adenosine phosphate compounds of rat tissues. By V. H. PARKER

Formation of formylkynurenine by the action of X-rays on tryptophan in aqueous solution. By G. G. JAYSON, G. SCHOLES and J. WEISS

Transglucosidation reactions with flavins. By L. G. WHITBY

Comparative detoxication. 3. Hippuric acid formation in adult locusts. By L. FRIEDLER and J. N. SMITH

Some observations on volatile fatty acids in the sheep's rumen. By E. F. ANNISON

Studies on the metabolism of semen. 9. Effect of surface active agents with special reference to the oxidation of succinate by spermatozoa. By H. H. KOEFOED-JOHNSEN and T. MANN

Two methods for the determination of glycogen in liver. By J. VAN DER VIES

The use of substances depressing antithrombin activity in the assay of prothrombin. By P. FANTL

Creatine phosphokinase: assay and application for the micro-determination of the adenine nucleotides. By J. B. CHAPPELL and S. V. PERRY

Creatine phosphokinase and the enzymic and contractile properties of the isolated myofibril. By S. V. PERRY

Studies in carotenogenesis. 12. The effect of temperature and thiamine concentration on carotenogenesis by *Phycomyces blakesleeanus*. By J. FRIEND and T. W. GOODWIN

Chemical analysis of human foetal skull bones. By I. MACDONALD

The use of paper partition chromatography for taxonomic studies of land snails. By R. L. KIRK, A. R. MAIN and F. G. BEYER

The effect of ketone bodies on the carbohydrate metabolism of rat diaphragm. By A. CHARI and E. WERTHEIMER

The component acids of the fats of some Indian fresh-water fishes. By S. P. PATHAK, G. D. PANDE and S. S. MATHUR

The organic constituents of mammalian compact bone. By J. E. EASTOE and B. EASTOE

Animal fats:

3. The component acids of ostrich fat. By F. D. GUNSTONE and W. C. RUSSELL
4. The component acids of crocodile fat. By F. D. GUNSTONE and W. C. RUSSELL

The sulphatase of ox liver. 3. Further observations on sulphatase *B* and an investigation of the origin of fractions *A* and *B*. By A. B. ROY

The interaction of proteins with silicic acid. By P. F. HOLT and J. E. L. BOWCOTT

Porphobilinogen. By G. H. COOKSON and C. RIMINGTON

The inhibition of growth and cozymase synthesis in bacteria by halogen-substituted nicotinic acids. By D. E. HUGHES

The regulation of respiration during the assimilation of nitrogen in *Torulopsis utilis*. By E. W. YEMM and B. F. FOLKES

The estimation of carbohydrates in plant extracts by anthrone. By E. W. YEMM and A. J. WILLIS

Separation of the bile pigments of serum, bile and urine. By P. G. COLE, G. H. LATHE and BARBARA H. BILLING

Polyol dehydrogenases. 1. The specificity of rat-liver polyol dehydrogenase. By JEAN MCCORKINDALE and N. L. EDSON

A sodium yeast and some of its properties. By E. J. CONWAY and P. T. MOORE

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **328th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University New Buildings, Edinburgh* 8, *on Friday, April 9th,* 1954, *at* 11 *a.m., when the following papers were read:*

COMMUNICATIONS

An Improved Technique for the Determination of Plasma Iron. By W. N. M. Ramsay. (*Department of Biochemistry, University of Edinburgh*)

The plasma-iron method previously published (Ramsay, 1953) suffers from the defect that it cannot be applied to perceptibly haemolysed specimens. It is also a potential source of error that the procedure involves filtration, and batches of Whatman no. 42 paper have been encountered which contain up to 0·5 μg. Fe/disk. While papers can be freed from iron by soaking in acetate buffer with dipyridyl and reducing agent, followed by washing with water and ethanol, it is an advantage that the modified technique, which eliminates the haemolysis error at least up to 800 μg. haem Fe/100 ml. plasma, permits filtration to be replaced by centrifugation except when very lipaemic specimens are encountered. The modifications consist in the use of sulphite instead of hydroxylamine as reducing agent, and in the use of a smaller quantity of buffer at a lower pH than before. 2 ml. plasma or serum is treated with 1 ml. acetate buffer, 0·5 M, pH 4·0, containing 2:2′-dipyridyl, 0·5 %, and 1 ml. sodium sulphite, 0·1 M. The volume is made up to 7·5 ml. and the tube heated 5–10 min. in a boiling water bath. The precipitate should be broken up with a glass rod about 1 min. before the tube is removed from the bath. After cooling, the mixture is either filtered through an iron-free paper or centrifuged. The optical measurement is conducted as before.

Figures are presented to show that the new technique gives results identical with the old one in unhaemolysed specimens, and that the addition of haemolysed cells introduces no error. It is worth noting that 'saccharated oxide of iron' (Slack & Wilkinson, 1949) is not quantitatively determined by either the sulphite or the hydroxylamine technique unless the heating period is extended to 1–1½ hr.

This work was supported by a grant from the Agricultural Research Council.

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Plasma Iron in a Terminal Case of Haemochromatosis. By Lynda M. H. Kerr and W. N. M. Ramsay. (*Department of Biochemistry, University of Edinburgh*)

Repeated determinations on six clinically established cases of haemochromatosis have given plasma-iron concentrations of 200–400 μg./100 ml., compared with normal values of 171 ± 37 (S.D.) (Ramsay, 1953). One of these, at the point of death from hepatic and cardiac complications, appeared to have a plasma iron of 1500 μg./100 ml. A serum determination shortly before death 24 hr. later gave a figure of 1460 μg./100 ml. Spontaneous occurrence of such high plasma-iron levels has not previously been recorded. A specimen of ascitic fluid obtained immediately after death showed an iron concentration of 290 μg./100 ml.

The serum was subjected to paper electrophoresis in glycine buffer at pH 8·6, and the paper was cut into strips. Separate pieces were used for (*a*) protein determination based on the reaction of Folin & Ciocalteu (1927) and (*b*) iron determination by an ultra-micro modification of the plasma-iron method. About 30 % of the iron was found in the β-globulin fraction and the remainder was fairly evenly distributed among the other fractions. In a haemochromatosis case with a plasma iron of 365 μg./100 ml., these other fractions, as in the normal, did not contain any analytically significant quantity of iron.

This work was supported by grants from the Agricultural and Medical Research Councils.

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Studies of Protein Biosynthesis. 1. A Method of Isolation and Identification of Peptides. By H. EDMUNDS and W. S. REITH*. (*Department of Botany, The University, Leeds*, 2)

In the course of work on protein synthesis in growing cells of plant roots (*Vicia faba*) we have worked out a method for the identification of peptides contained in various fractions of the tissue. It was carried out as follows: The deproteinized extracts were treated with DDPI (4-dimethylamino-3:5-dinitrophenyl-*iso*cyanate; Evans & Reith, 1954) as described for the preparation of DDP-ureidoalanylglycylglycine. The $NaHCO_3$ solution, containing the mixture of the DDP-ureidoacids of amino acids and peptides, was adjusted to pH 5·8 and was fractionated on a 4% cross-linked sulphonated polystyrene column with phosphate-citrate buffer pH 7.

Each separated zone was treated with 2N-HCl, when the *N*-terminal residue was split off as DDP-hydantoin, and the $(n-1)$-peptide was obtained.

The DDP-hydantoins being deeply orange-coloured, and soluble in a variety of organic solvents, can be easily separated from the $(n-1)$-peptides. By subsequent treatment of the hydantoins with $Ba(OH)_2$ the amino acids can be regenerated and identified by paper chromatography.

The various $(n-1)$-peptides can be hydrolysed by 6N-HCl and the hydrolysate analysed by paper chromatography. Alternatively, the $(n-1)$-peptides can be analysed by a method of amino acid sequence determination (Reith & Waldron, 1954).

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Observations on the Carbohydrase Activity of certain Seaweed Extracts. By W. A. M. DUNCAN, D. J. MANNERS and A. G. ROSS. (*Department of Chemistry, University of Edinburgh*)

By extraction of minced seaweed (from *Cladophora rupestris*, *Laminaria digitata* and *Rhodymenia palmata*) under weakly alkaline conditions, dilute solutions of proteins showing hydrolytic activity towards several carbohydrates were obtained. These carbohydrases have been concentrated by precipitation with ammonium sulphate and freeze-drying. Whole extracts from these seaweeds will hydrolyse certain α-glucosides (maltose, sucrose, αα-trehalose), several β-glucosides [cellobiose, 'cellodextrin' (an acid-degraded cellulose), laminaribiose, laminarin, barley β-glucosan, methyl β-glycoside, phenyl β-glucoside, amygdalin and salicin], lactose, xylotriose and the xylans from Esparto grass and *R. palmata*. No significant activity could be detected towards methyl α-glucoside, methyl α-galactoside, methyl α-mannoside, soluble starch, fucoidin, and inulin. Partial separation of these activities has been achieved by ammonium sulphate fractionation.

The 'maltase' present in the seaweeds shows both hydrolytic and synthetic activity; during enzyme action on maltose, glucose and small amounts of maltotriose and maltotetraose are formed. The maltase activity is unaffected by inorganic phosphate and is optimum at about pH 5·5.

During the hydrolysis of laminarin by a seaweed extract, glucose and a series of oligosaccharides were produced; the latter, on prolonged incubation, were hydrolysed to glucose. 'Cellodextrin' on incubation with seaweed extract yielded glucose and cellobiose during the initial stages of hydrolysis; the cellobiose was eventually hydrolysed to glucose. These results may indicate that the extracts contain a group specific β-glucosidase which can only hydrolyse terminal β-linked glucose units, and a second β-glucosidase capable of hydrolysing non-terminal β-glucosidic linkages. The seaweed β-glucosidases, unlike emulsin, do not show synthetic activity (cf. Peat, Whelan & Hinson, 1952); the 'laminarinase' activity is maximal at about pH 5·1.

The seaweed extracts show marked xylanase activity, the course of which is unaffected by inorganic phosphate. From the xylans from Esparto grass or *R. palmata*, xylose, xylobiose, xylotriose and higher saccharides are produced. It seems probable that the 1:3 linkages in the algal xylan are more easily hydrolysed than the 1:4 linkages (cf. Percival & Chanda, 1950).

We wish to thank Prof. E. L. Hirst, F.R.S., for his interest in this work which forms part of a research programme sponsored by the Institute of Seaweed Research.

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Further Observations on the Isolation and Chemical Composition of Castle's Intrinsic Factor. By A. L. LATNER, R. J. MERRILLS and LAUREEN C. D. P. RAINE. (*Section of Chemical Pathology, Department of Pathology, King's College, Newcastle upon Tyne*)

We have now succeeded in isolating a substance with high intrinsic factor activity without using a technique of preparative paper-strip electrophoresis (Latner, Ungley, Cox, McEvoy-Bowe & Raine, 1953). The activity has been demonstrated by studying the faecal excretion of orally administered radioactive vitamin B_{12} (Heinle, Welch, Meacham & Prusoff, 1952; Callender, Turnbull & Wakisaka, 1954).

The active material is extracted from a crude concentrate by means of a suitable buffer at pH 6·4 and impurities subsequently precipitated at pH 2·0 and 5·0. Electrolytes are removed by ultra-filtration and solid material obtained by freeze-drying. This material has proved to be as active as that originally obtained by preparative electrophoresis and is closely similar to it in chemical composition (Latner & McEvoy-Bowe, 1953). Both substances have proved essentially homogeneous when examined by paper-strip electrophoresis. Our newest material has been subjected to examination in the ultra-centrifuge by Dr R. A. Kekwick and has been shown to contain about 5% impurity of high molecular weight. The molecular weight of intrinsic factor material is below 20000.

The substance contains 10% nitrogen, 6·5% hexosamine, 2·2% fucose, 13·0% reducing sugar expressed as glucose, 1·04% tryptophan and 1·96% tyrosine. We now determine the hexosamine by a modification of the Elson and Morgan technique (Palmer, Smythe & Meyer, 1937). The presence of tyrosine has been demonstrated by ultraviolet absorption spectroscopy (Goodwin & Morton, 1946). The failure to find it previously was probably due to destruction during hydrolysis.

It is interesting that intrinsic factor possesses well marked blood-group substance activity in spite of the fact that the ultra-centrifuge data indicate absence of the usual blood-group substances.

We should like to thank Prof. W. T. J. Morgan for allowing one of us (R.J.M.) to carry out estimations of blood-group substance activity in his laboratory. Dr C. C. Ungley provided facilities for the radioactive vitamin B_{12} excretion tests and Dr F. T. Farmer and Mr C. G. Clayton supervised the counting apparatus. Our work is supported by a grant from the Medical Research Council.

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Permeability and 'Citridesmolase' Activity of *Aerobacter aerogenes*. By S. DAGLEY. (*Department of Biochemistry, University of Leeds*)

Dagley & Dawes (1953) showed that *Aerobacter aerogenes* harvested from a citrate-mineral salt medium was able to dissimilate citrate rapidly to acetate, CO_2 and pyruvate and that determinations of the latter, after 10 min. incubation of a standard suspension, provided a convenient measure of activity. The initial enzyme in dissimilation appears similar to that described by Gillespie & Gunsalus (1953) for *Streptococcus faecalis* and named by them 'citridesmolase'. Dagley & Dawes (1953) also showed that cells harvested during logarithmic growth at the expense of citrate, or from a medium in which citrate concentration limited the crop produced, were very active while those from a medium providing citrate in large excess were not. Bacteria from citrate-mineral salt media have now been crushed in a Hughes (1951) press and activities measured for suspensions of disintegrated cells at various dilutions. Linear relations, over a wide range, were established between initial rates of pyruvate production from citrate and the amount of suspension, expressed as total nitrogen (Kjeldahl). The activity per μg. N was the same whether the whole cells were active before crushing or whether they were inactive cells harvested from a medium in which excess citrate remained unconsumed. Whole cells from an aerated glucose-mineral salt medium were also inactive, but so were suspensions of disintegrated cells. The latter were able to oxidize, but not appreciably to dissimilate citrate.

Recent studies (Barrett, Larson & Kallio, 1953) have suggested that transport of a substrate to its site of action within a bacterial cell involves a carrier system that may be developed adaptively (Kogut & Podoski, 1953). Since whole cells which were unable to attack citrate at a measurable rate contained as much 'citridesmolase' as active cells,

loss of activity and cessation of growth in the presence of unconsumed citrate may be ascribed to impairment of the carrier mechanism by accumulating metabolic products. It appears that bacteria cannot always be assumed to be freely permeable to a substrate even when they have utilized it during growth.

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Dissimilation of Citrate by *Escherichia coli*. By S. Dagley. (*Department of Biochemistry, University of Leeds*)

Citrate utilization in complex media by *Escherichia coli* has been reported by Lominski, Conway, Harper & Rennie (1947) and by Vaughn, Osborne, Wedding, Tabachnick, Beisel & Braxton (1950). We find that in an unaerated medium containing mineral salts, trisodium citrate (5 g./l.), peptone and glucose (1 g./l.) whole cells, and preparations of disintegrated cells, were capable of producing pyruvate from citrate rapidly if they were removed from the medium when the glucose had been consumed. When the culture was allowed to stand, even at 4°, there was considerable loss of activity. This loss, for both whole and disintegrated cells, was rapid when active whole cells were incubated with a large volume of buffer; or, indeed, with the citrate-mineral salt medium that supports growth of *Aerobacter aerogenes*. Activity of such whole cells was rapidly restored on incubation with a peptone-citrate-glucose medium, but not when any one of these constituents was omitted. This effect appears to depend on the reconstitution or activation of the enzyme system since similar treatment of (inactive) cells grown in a glucose-peptone medium without citrate gave rise to little activity.

The nutritional conditions for enzyme development, and the loss of activity on transfer to a citrate-mineral salt medium, together indicate the inadequacy of views of citrate metabolism of *Escherichia coli* based solely on difficulties of cell permeability. We confirm that disintegrated cells from an aerated glucose medium will oxidize citrate while whole cells will not, but such preparations have little or no activity when tested without aeration by the method described above. Grunberg-Manago & Gunsalus (1953) have briefly reported an adaptive citrate fermentation pathway for *Esch. coli*. Our findings are similar to those of the other workers on formic hydrogenlyase development in *Esch. coli*, and in particular to those of Lascelles (1948). It may be significant that *Aerobacter aerogenes* does not require a complex medium to develop that enzyme (Dagley & Dawes, 1953).

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The Effect of *p*-Nitrophenol and of Arsenate on Aerobic Phosphorylation in Mammary Gland and Kidney Homogenates. By C. Terner. (*National Institute for Research in Dairying, University of Reading*)

Whole mammary-gland homogenates and washed kidney cortex suspensions of the guinea pig were incubated at 37° with reaction mixtures consisting of a basal medium (see Terner, 1954*a*) supplemented with fluoroacetate (0·005 M), fluoride (0·01 M), glucose and hexokinase. In this system, only a small uptake of inorganic phosphate was observed, which was enhanced by the addition of fumarate and further increased by the addition of pyruvate. In the absence and presence of fumarate, the uptake of phosphate was abolished by 2×10^{-4} M *p*-nitrophenol (*p*NP) which also caused some inhibition of respiration. In mammary gland homogenates incubated with fumarate plus pyruvate, 2×10^{-4} M-*p*NP caused up to 100% stimulation of respiration and citric acid synthesis (see Terner, 1954*b*), but only partly inhibited the accompanying phosphorylation. Higher concentrations of *p*NP (4×10^{-4} M) which abolished the phosphate uptake did not further increase the rates of respiration and citric acid formation. Similar observations were made with washed kidney homogenates except that in some of these preparations the stimulating effect of *p*NP on the respiration was small or absent.

Arsenate in low concentration (0·001–0·005 M) caused little or no inhibition of phosphorylation. Increasing amounts intensified the inhibition, but the effect was relatively small even in concentrations approximately equal to the initial phosphate content of the medium (0·01 M) and much larger amounts (0·02–0·04 M) had to be added to reduce the phosphate uptake to a minimum, confirming the findings of Crane & Lipmann (1953). Nevertheless, the presence of small amounts of arsenate made the phosphorylating system more susceptible to low concentrations of *p*NP; e.g. 0·005 M arsenate and 2×10^{-4} M-*p*NP which alone caused only a slight depression of phosphorylation, were in combination as strongly inhibitory as either 0·04 M arsenate or 4×10^{-4} M-*p*NP.

In contrast to *p*NP, arsenate in sufficiently high concentration (0·02 M) caused inhibition of respiration and citric acid synthesis. In its presence the stimulating effects of *p*NP were diminished but not abolished. In the present experiments the oxygen consumption remained almost linear in the presence of arsenate during incubation periods of 40–50 min., without the progressive inhibition of respiration which complicated the findings of Crane & Lipmann (1953).

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A Histochemical and Chromatographic Study of the Lipid Distribution in Human Arteries. By Anne E. Henderson and J. D. B. MacDougall. (*Departments of Biochemistry and Anatomy, University College, Dundee, University of St Andrews*)

A series of arteries was obtained from human subjects representing each decade from 0–90 years. The arteries studied were: brachial, which is rarely atheromatous, middle cerebral, left coronary, splenic and the aorta at four levels. The lipid distribution in the arterial wall was investigated by histochemical methods. The procedure followed was based on that of Cain (1949).

With increasing age there was an apparent increase in total lipid and cholesterol and its esters in all arteries, this being minimal in the brachial artery. In arteries showing atheroma, there was also an apparent increase in neutral fat and 'phospholipid'. Paper partition chromatography shows the presence of cholesterol, cholestane-3β:5α:6β-triol and cholest-5-ene-3β:7β-diol in the normal aorta.

We thank Dr R. P. Cook for his interest and the Advisory Committee on Medical Research (Scotland) for a grant.

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The Arylsulphatases of Mammalian Liver. By K. S. Dodgson, B. Spencer and J. Thomas. (*Physiology Institute, Newport Road, Cardiff*)

Fractionation of rat liver in isotonic sucrose (Schneider, 1948) and assay with dipotassium 2-hydroxy-5-nitrophenyl sulphate (0·04 M at pH 6·0) showed the arylsulphatase activity to be distributed thus: nuclear fraction 9, mitochondria 62, microsomes 21·5 and supernatant 16·5 %; total recovery 109 %. Assay of the same fractions with potassium *p*-acetylphenyl sulphate as substrate gave results in accord with previous findings (Dodgson, Spencer & Thomas, 1954), namely, 12·5, 6·0, 62 and 2·5 % respectively, total recovery 83·0 %. These figures suggested the presence, in rat liver, of two arylsulphatases possessing widely different affinities for the two substrates.

The presence of two arylsulphatases in acetone-dried rat liver was indicated by anomalous substrate concentration-activity curves for *p*-nitrophenyl sulphate although the curves for *p*-acetylphenyl and nitrocatechol sulphates appeared normal. A separation of the two enzymes was made by extracting the acetone-dried liver with 0·1 M acetate, pH 6·8, and centrifuging at 24000 *g* for 30 min. The resulting fractions showed widely different substrate concentration-activity curves whichever substrate was chosen. The 'debris enzyme' had a high affinity for *p*-nitrophenyl sulphate (K_m $4{\cdot}85 \times 10^{-4}$ M) whilst the 'supernatant enzyme' had a low affinity (K_m $1{\cdot}3 \times 10^{-1}$ M) towards this substrate. The difference in affinities of the two enzymes for *p*-acetylphenyl sulphate was of the same order but for nitrocatechol sulphate it was less marked. Similar findings were made for the acetone-dried livers of man, ox, mouse and rabbit but the ratio of the activities of 'debris' and 'supernatant' enzymes varied considerably with the species.

It is now clear that in previous work (Dodgson, Spencer & Thomas, 1953, 1954) assay of arylsulphatase activity with potassium *p*-acetylphenyl sulphate measured the activity of the 'debris' enzyme only, since under the conditions used (0·007 M substrate at pH 7·2) the activity of, and competition by, the 'supernatant' enzyme was negligible. On the other hand, although the 'supernatant' enzyme contributed the bulk of the arylsulphatase activity in the distribution studies when nitrocatechol sulphate was the substrate, the 'debris' enzyme had appreciable activity and competitive power. It is concluded that the 'debris' enzyme is responsible for the arylsulphatase activity of the microsomes whilst the mitochondria are the source of the 'supernatant' enzyme. Rupture of the mitochondria by acetone-drying or by alternate freezing and thawing releases the 'supernatant' enzyme in a soluble form whereas the microsomal enzyme remains insoluble.

There are indications that the 'supernatant' enzyme is a complex of two enzymes which might correspond to those found in ox liver by Roy (1953).

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The Effects of Analogues of Glutathione on the Glyoxalase Reaction. By W. O. Kermack and N. A. Matheson. (*Department of Biological Chemistry, University of Aberdeen*)

It is well established that the glyoxalase system consists of two enzymes, one of which, glyoxalase I, brings about the formation of a methylglyoxal-glutathione compound which is characterized by marked absorption at 240 mμ.; the other, glyoxalase II, catalyses the decomposition of this into lactic acid and glutathione (Hopkins & Morgan, 1948; Racker, 1951; Crook & Law, 1952).

In the complete glyoxalase system, glutathione cannot be replaced by cysteine but can be replaced by such analogues as α-L-glutamyl-L-cysteinylglycine and β-L-aspartyl-L-cysteinylglycine (Behrens, 1941) or γ-D-glutamyl-L-cysteinylglycine (Kogl & Akkerman, 1946).

The following compounds have been synthesized and their possible inhibitive action on glyoxalase I has been studied: γ-DL-glutamylglycylglycine, γ-DL-glutamyl-DL-alanylglycine, *S*-methyl-L-cysteine, *S*-methylglutathione, *S*-(*N*-ethylsuccinimido)-L-cysteine, *S*-(*N*-ethylsuccinimido)-glutathione and γ-L-glutamyl-L-cysteylglycine (the latter being a peptide containing cysteic acid). The action of glutamic acid and glycine on glyoxalase I was also studied. The formation of the methylglyoxal-glutathione compound was followed spectrophotometrically by observing the increase in optical density at 240 mμ. in a Unicam spectrophotometer (Racker, 1951).

The greatest inhibition has been obtained using *S*-(*N*-ethylsuccinimido)-glutathione but all the above compounds have inhibitory properties to some degree except *S*-(*N*-ethylsuccinimido)-L-cysteine, glutamic acid and glycine. With most of these inhibitors, the percentage inhibition depends mainly on the inhibitor concentration and varies little with the glutathione concentration so that it seems to be chiefly of the non-competitive type. These results have been controlled by experiments in which the activity of the complete enzyme system was measured by a manometric method (Woodward, 1935).

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Enzymes of Lobster Tissues. By W. O. Kermack, H. Lees and J. D. Wood. (*Department of Biological Chemistry, University of Aberdeen*)

Aerobic Warburg experiments under various conditions show that q_{O_2} (μl. O_2/mg. wet wt./hr.) for lobster muscle is less than 0·01. The corresponding values for hepatopancreas tissue are 0·08 at both 25° and 37°. The addition of magnesium, adenylic acid and cytochrome *c* to the lobster muscle had no effect, whilst for the hepatopancreas the q_{O_2} was increased by about 75 %, an effect which was found to depend on cytochrome *c*.

The addition of *p*-phenylenediamine alone caused only a small increase in q_{O_2}, but in the presence of cytochrome *c* the q_{O_2} increased to 0·54, from which it appeared that the hepatopancreas contained cytochrome oxidase.

Various possible metabolites, including most of the components of the citric acid cycle, had no effect on the q_{O_2} of muscle either with or without the addition of magnesium, adenylic acid and cytochrome *c*. Similar negative effects were obtained with hepatopancreas tissue except on the addition of acetaldehyde, methylglyoxal, and other aldehydes. The aldehyde oxidase activity of this tissue showed many resemblances to that of pig liver (Gordon, Green & Subrahmanyan, 1940). With acetaldehyde at pH 7·1, the total oxygen uptake was less than the theoretical but at pH 5·3 the total oxygen uptake was 99 % of theory.

The addition of quinine failed to increase the q_{O_2} of hepatopancreas tissue. It would therefore appear that unlike mammalian liver aldehyde oxidase the lobster hepatopancreas enzyme does not oxidize quinine (Knox, 1946).

The addition of azide increased the q_{O_2} at pH 7·1, but it had no effect at pH 5·3 unless present in large quantities when a decrease in the q_{O_2} was observed.

It is known that the tissues of certain invertebrates contain glyoxalase, as detected by the formation of acid from methylglyoxal in presence of glutathione (Hopkins & Morgan, 1945). We have now shown that glyoxalase is present in lobster muscle ($q_{CO_2} = 0{\cdot}85$) and also in the hepatopancreas ($q_{CO_2} = 1{\cdot}34$).

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Separation of the Phospholipids of Egg Yolk on Silicic Acid. By C. H. Lea and D. N. Rhodes.
(*Low Temperature Station, University of Cambridge and Department of Scientific and Industrial Research*)

Egg-yolk phospholipid purified by rejection of material insoluble in cold ether and by removal of contaminating amino acids (Lea & Rhodes, 1953) was applied, in methanol-chloroform solution (25 %, v/v), to thick filter paper impregnated with silicic acid (Kirchner & Keller, 1950) and activated at 120°. A fast-moving amino-containing (ninhydrin reaction) fraction ($R_F \sim 0{\cdot}9$) was well separated from slower-moving choline-containing (phosphomolybdate reaction) fractions ($R_F \sim 0{\cdot}6$ and $\sim 0{\cdot}1$). This separation of phosphatidylethanolamine, lecithin and lysolecithin was better than anything we have been able to achieve to date on untreated paper.

The phospholipids (1 g.) also separated when applied to a column of silicic acid (24 g., activated at 120°, with 6 g. acid-washed Celite), eluting with a suitable concentration of methanol in chloroform (e.g. 15–30 %, v/v, according to the activity of the adsorbent) under a slight pressure of nitrogen. Progress of the phospholipids through the column was followed by direct determination of phosphatidylethanolamine without hydrolysis (Lea & Rhodes, 1954) and of total P in the eluate.

The whole of the amino-containing phospholipid was recovered in a fast-running, cleanly separated fraction, shown by paper chromatography of a hydrolysate to be free from choline. The main choline-containing fraction which followed gave no ninhydrin reaction and possessed the correct N/P ratio, P content and fatty acid/P ratio for lecithin. Part of the residue on the column after removal of the lecithin could be eluted with methanol and from its haemolytic activity, insolubility in ether and R_F value on silica paper appeared to be lysolecithin.

Many preparations of egg phospholipids obtained by different extraction procedures, as well as 'lecithins' prepared from them by the alumina column (Hanahan, 1951) and cadmium chloride (Pangborn, 1951) methods, have been found to give low fatty acid/P ratios. This could be due to the presence of lysophospholipids and the question as to whether these substances are normal constituents of egg yolk or arise during the extraction procedure is under investigation.

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Enzymic Cleavage of Anserine in Skeletal Muscle of Codling (*Gadus callarias*). By N. R. Jones. (*Department of Scientific and Industrial Research, Torry Research Station, Aberdeen*)

Gutted codling were iced, and extracts of muscle prepared and chromatographed as described previously (Jones, 1954). Serial chromatograms showed increasing free L-1-methylhistidine and β-alanine contents during the earliest spoilage phase, before invasion of the tissue by bacteria. This increase was also found at 0° and 15° in sterile, lysing muscle homogenates and cell-free aqueous muscle extracts. Subsequently the two amino acids were isolated from muscle autolysates by separation on ion-exchange resin columns.

It seemed likely that these amino acids derived from anserine (β-alanyl-L-1-methylhistidine) which is present in codling muscle to the exclusion of the related dipeptide carnosine (Yudaev, 1950; Shewan, 1954). Incubation of added anserine with aqueous muscle homogenates or cell-free extracts led to increased β-alanine and L-1-methylhistidine formation in systems buffered at pH 7·0 with 0·05M phosphate or 0·03M veronal.

Anserine-cleaving activity of extracts did not pass a membrane during dialysis against water. Much material of high molecular weight was precipitated under these conditions without loss of activity in solution. The dialysed preparation was free from substrate and products. It was stable to freeze-drying and could also be concentrated under reduced pressure at an external temperature of 35° without appreciable loss of activity. Extracts of acetone powders, prepared from muscle homogenates at low temperatures, were also active.

Complete loss of activity resulted from heating preparations at 100° for 1 min. in the presence or absence of neutral salts or substrate. Acid precipitation similarly destroyed activity.

The properties of the anserine-cleavage factor indicate that it is an enzyme, for which the name 'anserinase' is suggested. This follows the precedent of Hanson & Smith (1949), who confirmed the presence of a swine kidney 'carnosinase' previously noted in crude kidney extracts by Garkovi (1937), and Abderhalden & Parshin (1947). Carnosinase activity has not been demonstrated in muscle although various workers have examined the tissue (Hanson & Smith, 1949). The writer is not aware of previous reports of anserinase activity in muscle but Yudaev (1952) has reported *in vivo synthesis* of anserine from the constituent amino acids by the rabbit.

This work was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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The Characterization of Histones from Calf-Thymus Nucleoprotein. By J. A. V. Butler, P. F. Davison, D. W. F. James and K. V. Shooter. (*Chester Beatty Research Institute, London, S.W.* 3)

Histones extracted from calf-thymus nucleoprotein by dilute acids have been studied by physical methods and a partial fractionation achieved. The nucleoprotein preparations were extracted at low ionic strength (Butler, Davison, James & Shooter, 1954).

Electrophoresis reveals two components below pH 7 but three above. Grégoire, Grégoire & Reynaud (1953), who worked only at pH's above neutrality, have also found three components. However, it is possible that the middle electrophoretic boundary represents a complex of the other two proteins, which aggregate as the isoelectric point is approached. A preparation containing two components in equal proportions at pH 4·5, contains 70% of the material as the middle component at pH 8·5. One component, when isolated, remained homogeneous throughout this pH range investigated.

Observations in the ultracentrifuge suggest that at least one of the proteins forms dimers and larger aggregates. Variable amounts of a fast component (S_{20} about 10S) are present, but this disappears as the pH is lowered. At and below pH 4·5 the sedimentation diagram reveals an asymmetric peak which has been tentatively resolved into two components with S_{20} close to 1S and 2S. The proportion of the lighter component increases as the pH is lowered from 4·5 to 1. The fact that the electrophoretic picture does not change below pH 7 strongly suggests that these aggregates are formed only between similar molecules.

A partial fractionation has been achieved by treatments with acetone and ammonia. The more soluble fraction, corresponding to the slower electrophoretic boundary is homogeneous under electrophoresis, and apparently so in the ultracentrifuge ($S_{20} = 0{\cdot}975$). The diffusion constant was $5{\cdot}3 \times 10^{-7}$. Successive analysis showed the diffusion curve to be Gaussian. This protein has a molecular weight of 18000, and an axial ratio of 27:1. The high axial ratio is not unexpected as the whole histone slowly gels above pH 8. This component has a much lower tyrosine content than the parent histone preparation, which is in agreement with Stedman & Stedman's earlier fractionation and analysis (1950).

The maximum tryptophan content in the whole histone was found to be 0·1%. One residue per molecule would require a protein of molecular weight 200000, and these and previous measurements (Butler, Davison, James & Shooter, 1954) make it certain that neither of the major components has nearly this size. Thus the tryptophan must be ascribed to a minor constituent.

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FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal:*

Trypsin-catalysed transpeptidations. By S. G. Waley and J. Watson

Chymotrypsin-catalysed transpeptidations. By K. Blau and S. G. Waley

The absorption of methionine by the skin of the guinea pig. By L. J. Edwards

The effect of ammonia on the oxidation of glucose by *Zygorhyncus moelleri*. By V. Moses

Studies on yeast metabolism:

3. The intracellular level of pyruvate during yeast fermentation. By W. E. Trevelyan and J. S. Harrison
4. The effect of thiamine on yeast fermentation. By W. E. Trevelyan and J. S. Harrison

The estimation of purines in nucleic acids: a method applicable to materials with low concentration of nucleic acid. By I. W. McDonald

The oxidation of myoglobin to metmyoglobin by oxygen. 3. Kinetic studies in the presence of carbon monoxide, and at different hydrogen-ion concentrations with considerations regarding the stability of oxymyoglobin. By P. George and C. J. Stratmann

South African pilchard oil:

2. Concentrates of highly unsaturated fatty acids and alcohols derived from South African pilchard oil. By M. H. Silk, H. H. Sephton and H. H. Hahn
3. The fatty acid composition of South African pilchard oil. By M. H. Silk and H. H. Hahn
4. The isolation and structure of a hexadecatetraenoic acid from South African pilchard oil. By M. H. Silk and H. H. Hahn

Studies in the biochemistry of micro-organisms. 93. Cyclopenin, a nitrogen-containing metabolic product of *Penicillium cyclopium* Westling. By A. Bracken, Anna Pocker and H. Raistrick

The purification of alkaline phosphatases of animal tissues. By R. K. Morton

Copper complexes in grassland herbage. By C. F. Mills

The lipids of fish. 5. The lipids remaining in the flesh of the haddock after extraction by acetone and ethanol-ether. By June Olley and J. A. Lovern

The reaction of haemoglobin and some of its derivatives with *p*-iodophenylhydroxylamine and *p*-iodonitrosobenzene. By H. Jackson and R. Thompson

The interaction of dyes with proteins on paper with special reference to paper electrophoresis. By G. T. Franglen and N. H. Martin

Studies on the biosynthesis of riboflavin. Nitrogen metabolism and flavinogenesis in *Eremothecium ashbyii*. By T. W. Goodwin and S. Pendlington

On the significance of γ-glutamyl transpeptidation in peptide biosynthesis. By R. W. Hendler and D. M. Greenberg

Techniques in tissue metabolism. 3. Study of tissue fragments with little or no added aqueous phase, and in oils. By R. Rodnight and H. McIlwain

Appendix—Manometric determination of the solubility of oxygen in liquid paraffin, olive oil and silicone fluids. By R. Rodnight

Electrical pulses and the metabolism of cell-free cerebral preparations. By A. Narayanaswami and H. McIlwain

Applied electrical pulses and the ammonia and acetylcholine of isolated cerebral cortex slices. By E. V. Rowsell

Rapid changes in creatine phosphate level in cerebral cortex slices. By P. J. Heald

The action of some ergot derivatives, mescaline and dibenamine on the metabolism of separated mammalian cerebral tissues. By Joyce L. Lewis and H. McIlwain

The metabolism of short-chain fatty acids in the sheep. 3. Formic, *n*-valeric and some branched-chain acids. By E. F. Annison and R. J. Pennington

Some esterases of the rat. By W. N. Aldridge

A study of some simple peptide complexes with zinc and cadmium ions in aqueous solution. By D. J. Perkins

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **329th Meeting** *of the Biochemical Society was held in the Department of Physiology, The University, Bristol* 8, *on Saturday,* 15 *May* 1954, *at* 11 *a.m., when the following papers were read:*

COMMUNICATIONS

Further Observations on the Biosynthesis of Riboflavin by *Eremothecium ashbyii*. By E. G. Brown, T. W. Goodwin and S. Pendlington. (*Department of Biochemistry, The University of Liverpool*)

Goodwin & Pendlington (1954) showed that of all the amino acids they examined only serine and threonine specifically stimulated riboflavin production in *Eremothecium ashbyii*; furthermore, the purines adenine and xanthine also stimulated flavinogenesis, although the naturally occurring pyrimidines did not.

It has now been found that, under the same experimental conditions, neither 5:6-dimethylbenziminazole nor 1:2-dimethyl-4-amino-5-(D-1′-ribityl)-aminobenzene increases flavinogenesis; there is, in fact, slight inhibition. These results tend to confirm the previous conclusion that a purine or a close derivative is the precursor of the 'nitrogen-containing residue' of riboflavin. Both the above compounds inhibit the threonine stimulation considerably but have little effect on the adenine stimulation. A mixture of 1:2-dimethyl-4-amino-5-(D-1′-ribityl)-aminobenzene and alloxan appears slightly to increase riboflavin synthesis, but the situation is complicated by the spontaneous production of a mixture of riboflavin and a second yellow compound when these two substances are mixed in the absence of *E. ashbyii*.

Over the concentration range 0·002–0·2 mg./100 ml., 4-aminopterin inhibits growth of *E. ashbyii*, whilst at the same time enhancing flavinogenesis; at 0·2 mg./100 ml. inhibition of growth is almost complete; this effect is not overcome by folic acid, but it is counteracted by increasing the peptone concentration in the medium. 4-Aminopterin also inhibits both the threonine and adenine stimulation, the latter to a less extent.

When glucose (1 %, w/v) is replaced by the same concentration of acetate, growth is reduced by about 70 %, whilst the production of riboflavin in both mycelium and medium is negligible. Increasing the acetate concentration to 2 % (w/v) increases growth but does not stimulate riboflavin synthesis. No growth occurred on a medium containing 4 % (w/v) acetate. Resuspension of washed 'acetate mycelia' in glucose + adenine or glucose alone resulted in only slight riboflavin synthesis.

Previous work suggested that the threonine and serine stimulation might be due to the provision by these amino acids of non-nitrogenous precursors of the aromatic ring of riboflavin. Of the possible precursors, examined at a concentration of 25 mg./100 ml., only pyruvate was found consistently to stimulate riboflavin synthesis, although to a lesser degree than serine or threonine; β-hydroxybutyrate and α-oxobutyrate were without obvious effect; acetate was slightly inhibitory, whilst α:β-dihydroxybutyrate, crotonate and *n*-butyrate were more inhibitory. The pyruvate and adenine stimulations were additive whilst those of pyruvate and threonine were not.

We wish to thank Dr E. L. Holdsworth, National Institute for Research in Dairying; Dr J. R. Ruegsegger, The Lederle Laboratories; and Dr V. Petrow, British Drug Houses Ltd, for generous gifts of chemicals.

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The Metabolism of Naphthalene in Different Animal Species. By E. D. S. Corner and L. Young. (*Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E.* 1)

The metabolism of naphthalene in animals has been reviewed by Young (1950), and more recent work has been described by Boyland & Wiltshire (1953), Boyland & Solomon (1953) and Corner, Billett & Young (1954).

The present investigation was undertaken in order to gain information concerning the nature of the hydroxyl derivatives, both free and conjugated, which are excreted by animals dosed with naphthalene. Because it is known that rabbits and rats are able to metabolize naphthalene in different ways, both these species were studied in the present investigation, as also were guinea pigs and mice.

In the course of preliminary work using paper chromatography, the urines of rabbits and rats dosed with naphthalene were found to contain

1:2-dihydronaphthalene-1:2-diol, 1- and 2-naphthol, 1-naphthylglucosiduronic acid, and 1-naphthylsulphuric acid. Guinea pigs dosed with naphthalene excreted 1- and 2-naphthol in approximately equal amounts, 1:2-dihydronaphthalene-1:2-diol and traces of 1:2-dihydroxynaphthalene and 1-naphthylsulphuric acid. Urine from mice dosed with napthalene contained 1-naphthol, and small amounts of 1:2-dihydronaphthalene-1:2-diol, 2-naphthol, 1-naphthylglucosiduronic acid and 1-naphthylsulphuric acid.

The following compounds were isolated from the urines of the dosed animals. In the case of rabbits a small quantity of 2-naphthol (m.p. 121–122°; acetate, m.p. 70–70·5°) was isolated in amounts corresponding to 0·5 % of the naphthalene administered. (−)-*trans*-1:2-Dihydronaphthalene-1:2-diol, m.p. 126–127°, $[\alpha]_D^{18°} - 157°$ in ethanol (*c*, 1), was isolated from the urine of guinea pigs in an amount corresponding to 1 % of the hydrocarbon injected; and a small amount of 1-naphthol (m.p. 93–94°; acetate, m.p. 48–49°) representing 0·3 % of the dose was separated from the urine of mice dosed with naphthalene.

It has been shown that rabbits convert naphthalene into 1:2-dihydro-2-hydroxy-1-naphthylglucosiduronic acid (Corner *et al.* 1954). In the present work evidence was obtained that this compound is also a metabolite of naphthalene in the rat. A glucosiduronic acid fraction was separated from the urine of rats dosed with naphthalene and it was found that incubation of this fraction with β-glucuronidase led to the liberation of 1:2-dihydronaphthalene-1:2-diol.

Naphthalene is known to be converted into 1-naphthylmercapturic acid in rabbits (Bourne & Young, 1934) and rats (Young, 1947). This compound (m.p. 170–171°) has now been isolated from the urines of mice and guinea pigs in amounts corresponding to 6 % of the hydrocarbon administered to mice and 0·6 % of the dose injected into guinea pigs.

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Some Observations on the Composition, Function and Biogenesis of Plant Gums with Particular Reference to the Plum Gums. By J. B. Pridham (introduced by C. A. Ashford). (*The Biological Chemistry Laboratory, University of Bristol*)

The gum that is exuded from the bark and fruit of plum trees, like other plant gums, consists largely of a complex acidic polysaccharide composed of D-glucuronic acid, D-galactose, D-mannose, L-arabinose, D-xylose and L-rhamnose (cf. Hirst & Jones, 1947).

It has been suggested (Hirst & Jones, 1951) that gummosis is a protective mechanism which enables the plant to seal off lesions made by mechanical injury or invading micro-organisms. Small-scale experiments indicate that gum formation is probably enhanced when micro-organisms are introduced artificially into plum trees, and repeated failures to degrade gums *in vitro* with micro-organisms illustrate the value of these compounds to the tree.

Apart from the large carbohydrate fraction, the exuded gums also contain small amounts of phenolic compounds, lignin and protein, and show peroxidase activity, as do gum arabic (Whistler & Smart, 1953), gum *Cedrela sinensis*, and grapefruit gum.

The proportions of D-galactose, D-mannose, L-arabinose and D-xylose, after hydrolysis, in Victoria plum bark and fruit gums, have been determined by the benzidine method (Jones & Pridham, 1953), and it has been shown that the bark gum contains a higher proportion of D-mannose and L-arabinose than the fruit gum. Other properties, however, such as optical rotation and rate of hydrolysis, are similar for both gums.

The use of the Tiselius electrophoresis apparatus for investigating the homogeneity of water-soluble polysaccharides has been explored, and artificial mixtures of gum arabic and plum gum in borate buffer (pH 7·8), and sugar-beet araban and plum gum in phosphate buffer (pH 7·0) have been sucessfully separated.

All attempts to fractionate bark and fruit gums electrophoretically, either singly or using an artificial mixture of the two, have so far failed.

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Effect of Acetate on Glycogen Synthesis and Glucose Utilization in the Isolated Rat Diaphragm.
By R. J. GARNER and R. ROBERTS. (*Veterinary Laboratory, University of Bristol and Department of Biochemistry, University of Liverpool*)

Parnes & Wertheimer (1950) reported that in media containing 100 mg. % Na acetate in addition to glucose in concentrations of 100 mg. % or less, glycogen synthesis in diaphragms from rats fed on a stock diet was increased to a greater extent than would be expected from the sum of synthesis values for each substrate alone; glucose utilization and oxidation were also depressed. These experiments have been repeated using diaphragms from rats fed on diets containing 56 % starch, 10 % fat and 23 % casein ('high carbohydrate') and 10 % starch, 56 % fat and 23 % casein ('high fat'), the incubation medium containing either 0·08 % glucose alone or with 0·1 % Na acetate.

Contrary to the findings of Parnes & Wertheimer, acetate had little effect on glycogen synthesis in the 'high carbohydrate' diaphragms, whereas in diaphragms from fat-fed animals it produced a significant increase ($P < 0.01$), the high fat diet apparently inducing a relative inability of the diaphragms to synthesize glycogen except in the presence of acetate. Acetate in a diaphragm adapted to carbohydrate depressed glucose utilization slightly, but stimulated this utilization in 'high-fat' diaphragms. The rate of oxygen uptake in both groups was stimulated by acetate. Na butyrate had a similar effect to acetate on glycogen synthesis and glucose utilization in diaphragms from rats on the high carbohydrate diet.

A number of experiments were also carried out using glucose uniformly labelled with ^{14}C. These confirmed the earlier findings, but one somewhat surprising feature emerged. Inclusion of acetate produced a significant decrease in the amount of glucose being incorporated into glycogen in all diaphragms and, in the 'high-fat' diaphragms only, a significant increase ($P < 0.01$) in the amount of labelling appearing in the CO_2. This could indicate a preferential oxidation of glucose, but is contrary to what would be expected if acetate exhibited a sparing action of the type described by Parnes & Wertheimer or if, as suggested by Drury & Wick (1953), acetate is oxidized in preference to glucose.

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Haemoglobin in Certain Strains of Yeast (*Saccharomyces cerevisiae*). By D. KEILIN and A. TISSIÈRES. (*Molteno Institute, University of Cambridge*)

The occurrence of haemoglobin in yeast cells was so far recorded in only a few samples of commercial baker's yeast (Keilin, 1953). Haemoglobin was also found in *Penicillium notatum* and *Neurospora crassa*, especially in some of the slow-growing mutants of the latter (Keilin & Tissières, 1953). We are now able to extend these observations to several well-determined strains of yeast available in pure cultures.

Of several strains of yeast we examined, haemoglobin was detected in strains 67 and 77 of the Institute of Brewing and in a strain we isolated from top brewer's yeast of a local brewery (Greene, King and Sons).

Yeast suspensions of these strains on standing show the absorption bands of reduced cytochromes *a*, *b* and *c*. On aeration these bands are replaced by a band at 583 mμ which, however, rapidly disappears when aeration is interrupted and the bands of the reduced cytochrome begin to reappear. In presence of cyanide these bands can be seen together with the band of 583 mμ which disappears only in the presence of $Na_2S_2O_4$ or CO. These experiments, like those previously described (Keilin, 1953), indicate that the band at 583 mμ. belongs to oxyhaemoglobin.

From strain 77 ($Q_{O_2} = -82$, $Q^{air}_{CO_2} = +147$) referred to above and strain 79 ($Q_{O_2} = -72$, $Q^{air}_{CO_2} = +100$) which failed to show haemoglobin, the slow growing, 'petite colonie' strains 77A and 79A were isolated after cultivation in presence of acriflavine according to Ephrussi, Hottinguer & Chimènes (1949).

The strains 77A ($Q_{O_2} = -5$, $Q^{air}_{CO_2} = +250$) and 79A ($Q_{O_2} = -7$, $Q^{air}_{CO_2} = +240$) are found to be rich in cytochrome *c* but devoid of cytochromes *a* and *b*. They also show a very distinct band at 583 mμ., but, since the respiration of these strains is very low, this band can be seen together with those of reduced cytochrome *c*, which do not readily disappear on aeration. However, on addition of $Na_2S_2O_4$ the band at 583 mμ. disappears and on treatment with CO it is replaced by a diffuse band at 575 mμ.

The band at 583 mμ. in strains 77A and 79A therefore belongs to oxyhaemoglobin, the concentration of which, determined by matching it with

that of a standard solution of oxymyoglobin, was found to be 0·114–0·127 % of the dry weight of yeast (cf. Keilin & Ryley, 1953).

Since the conditions for the development of strains 77A and 79A, the magnitude of their respiration and fermentation and the structure of their cytochromes resemble those of the 'petite colonie' mutants studied by Slonimski (1949), Slonimski & Ephrussi (1949), Ephrussi (1952) and Slonimski (1953), it is conceivable that the band at about 580 mμ. recorded by these workers may also belong to oxyhaemoglobin.

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The Photochemical Inactivation and Microbial Metabolism of the Chlorophenoxyacetic Acid Herbicides. By W. C. Evans and B. S. W. Smith. (*Department of Agricultural Chemistry, University College of North Wales, Bangor*)

Carrol (1949), and Hansen & Buchholtz (1952) showed that riboflavin under the action of light, inactivated 2:4-dichlorophenoxyacetic acid (2:4-D.) in aqueous solution, both in the presence and absence of living plant tissues. Evidence was presented by the latter workers that one of the products of this photochemical action was a phenol—possibly 2:4-dichlorophenol (smell, reaction with bromine). We have now shown that in the *in vitro* reaction, *p*-chlorophenoxyacetic acid and 2:4-D. give rise to *p*-chlorophenol and 2:4-dichlorophenol respectively, which were isolated as benzoates. From paper chromatographic evidence, the production of glyoxylic and a trace of glycollic acid was also inferred.

The disappearance of 2:4-D. in soil (Nutman, Thornton & Quastel, 1945; Audus, 1949, 1950; Jensen & Petersen, 1952) has been attributed to its metabolism by organisms typed as members of *Bacterium globiforme* group, *Flavobacterium aquatile* and a *Corynebacterium*-like organism. These organisms grew poorly or not at all in simplified liquid media containing the herbicide, and work on its metabolic pathway has been hampered. By elective culture methods we have isolated from soil a small, Gram-negative, motile organism, which grows freely under aerobic conditions in a mineral salt medium containing *p*-chlorophenoxyacetic acid (up to 0·1 %) as sole source of organic carbon. From these cultures 2-hydroxy-4-chlorophenoxyacetic acid and 4-chlorocatechol have been isolated. These presumed intermediates satisfy the criteria of simultaneous adaptation with cells grown on *p*-chlorophenoxyacetic acid as initial substrate. Incubation of 4-chlorocatechol with these cells leads to ring fission, with the production of chloride ions and organic acids.

A small Gram-negative motile rod was also isolated from litter under conifers which grows in a 2:4-D. (up to 0·1 %) mineral salt medium, after an initial lag period. From such cultures we have detected a phenolic acid giving similar colour reactions to 2-hydroxy-4-chlorophenoxyacetic acid but a slightly different R_F value on chromatography. It is suggested that this phenolic acid metabolite of 2:4-D. is 6-hydroxy-2:4-dichlorophenoxyacetic acid.

In the bacterial metabolism of the chlorophenoxyacetic acids it would seem that the chlorine radicals are not eliminated from the molecule until it has lost its aromatic character. Their metabolic pathway presents analogous features to that already elucidated by Evans, Smith, Linstead & Elvidge (1951) for simpler aromatic compounds. That mammalian tissues (e.g. liver) contain systems capable of liberating chloride ions from aliphatic chloro compounds has recently been confirmed by Bray, Thorpe & Vallance (1952), who also demonstrated the participation of SH compounds in this reaction.

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Purification and Properties of Arginase. By S. J. Bach and D. B. Whitehouse. (*Department of Physiology, University of Bristol*)

The enzyme was purified from ox liver. The method used was essentially identical with that described by Bach (1946) in which the difficulty of removing the liver pigments had been overcome by their precipitation with $ZnSO_4$ at a concentration of 0·025% (w/v). Subsequent ammonium sulphate fractionation led to a colourless, highly active solution of the enzyme, the purity of which was demonstrated by the appearance of a single peak during electrophoresis. The Q_{urea} of such preparations was approximately 60 000 at 30°. The absorption spectra of the enzyme preparation at pH 7·0 were studied in the different stages of the purification; with the final solution only two bands (at 2200 Å and 2800 Å) were observed.

Investigations of the pure enzyme preparation in presence of high substrate concentrations showed the expected linear relationship between activity on the one hand and the reaction time or enzyme concentration on the other. The pH optimum was found to lie between pH 9·0 and 10·0. The K_m in metal-free solutions was found to be $5{\cdot}75 \times 10^{-3}$ M.

Of the metals tested at a concentration of 4×10^{-2}M only Mn^{2+} ions exerted a strongly activating effect on highly purified enzyme preparations. Since the activity of such solutions was considerably reduced after exhaustive dialysis against ethylenediaminetetraacetic acid (Versene) and could be fully restored by subsequent 24 hr. incubation with Mn^{2+} ions (at 20° and at pH 7·5), the latter appear to represent an essential component of the enzyme. While in this way the activating effect of Mn^{2+} ions, as reported by other workers (Greenberg, 1951; Roche, 1953) was confirmed, similar statements concerning the activating effect of cobalt ions could not be substantiated under the above experimental conditions. If, however, enzyme preparations of a lesser degree of purity were subjected to cobalt treatment, the arginase activity was considerably increased.

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The Inhibitory Effect of β-Amino*iso*butyric Acid on the Growth of Yeast. By Rosemary Lindan (introduced by O. Lindan). (*The Medical Research Council, Human Nutrition Research Unit, Holly Hill, London, N.W.* 3)

Pollack (1943), investigating the effects of α-methyl analogues of β-alanine and pantothenic acid on the growth of Gebruder-Mayer yeast, reported that β-amino*iso*butyric acid (BAIBA) possessed a growth-promoting effect similar to β-alanine, the relative potency, however, being only 0·0006. When BAIBA was added in the presence of β-alanine it showed a slight repressing action on the growth effect of the latter, a minimum growth point being reached at a concentration of 0·8 μg. BAIBA/ml. Concentrations of BAIBA above this level again caused an increase in the growth of the yeast. Pollack employed β-alanine at a constant level. His basal media contained also aspartic acid, which Hartelius (1946) showed could itself cause inhibition of yeast growth when added to a medium in which the concentration of β-alanine was suboptimal.

In the present experiments a simple basal medium was used, in which ammonium sulphate formed the nitrogen source. The strain of yeast employed was *Saccharomyces carlsbergensis* 4228, for which β-alanine is a growth stimulant but not an essential nutrient. It was found that the addition of BAIBA to the basal medium caused appreciable depression of yeast growth. At a level of 40 μg./ml. growth was depressed by about 50% and 160 μg./ml. depressed it by over 90%. No evidence was obtained of any growth stimulatory effect, using concentrations of BAIBA ranging from 0·08 to 8000 μg./ml. The inhibitory effect was less marked when β-alanine was also added to the medium, and became progressively less as the concentration of β-alanine was raised. When 2 mg./ml. of calcium(+)-pantothenate was added to the basal medium no inhibition of yeast growth was detectable using BAIBA up to a concentration of 160 μg./ml. Some inhibition could still be produced however if β-alanine were also added, and this inhibition became more marked when the pantothenate level was lowed to 1 mg./ml. From these results it would seem that the inhibitory action of BAIBA on the growth of the examined yeast is due to the formation of α-methylpantothenic acid which interferes with pantothenic acid utilization.

I should like to thank Dr J. Done for supplying me with his synthetic preparation of β-amino*iso*butyric acid.

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Carbohydrate and Amino Acid Constituents of the Cell Walls of *Corynebacterium diphtheriae*. By C. S. CUMMINS and H. HARRIS. (*Departments of Bacteriology and Biochemistry, London Hospital Medical College, E.* 1)

Cell-wall material has been prepared from *Gravis*, *Mitis* and *Intermedius* strains of *Corynebacterium diphtheriae* by disintegration with small glass beads and digestion of the particulate fraction with crude trypsin. Electron micrographs show that such material is almost entirely composed of cell-wall fragments.

In a *Mitis* strain such material was shown to contain at least two antigens (Cummins, 1954); a specific protein antigen, and a group antigen, apparently polysaccharide, found in strains of all three types. The specific antigen is destroyed by pepsin, and pepsin-treated cell-wall suspensions appear to contain only group antigen. This group antigen is inactivated by 0·01M periodate at pH 7 in 2 hr. at 18–20°.

Trypsin-purified, pepsin-treated cell-wall material was hydrolysed in 6N-HCl for 8 hr. at 100°, and the amino acids examined by two-dimensional chromatography (phenol/lutidine). Alanine, glutamic acid and diaminopimelic acid were present in very much larger amounts than any other ninhydrin-reacting material. Leucine, valine, threonine, glycine, serine, aspartic acid and lysine were also detected.

Besides the amino acids, two ninhydrin-reacting substances were constantly present, which in duplicate chromatograms developed by Partridge's modification of Morgan and Elson's method (Partridge, 1948), gave cherry red spots. One of these corresponds to glucosamine, the other has not been identified but is probably not chondrosamine since it moves more slowly than glucosamine in phenol, whereas chondrosamine moves more rapidly.

Samples of the material from strains of all three types hydrolysed in 2N-H_2SO_4 for 2 hr. at 100°, and run two-dimensionally in phenol/lutidine, constantly showed the presence of arabinose, galactose and mannose, in that order of concentration.

Since the group antigen is inactivated by periodate, samples were hydrolysed after treatment with 0·01M periodate for 2 hr. and examined by chromatography. The amino acids and hexosamines were relatively unaffected, arabinose and galactose were apparently reduced to about 50–70% of their former value, but mannose appeared to be more rapidly attacked and was diminished to about 15–30%. Even after 24 hr. treatment, appreciable amounts of arabinose and galactose could still be detected.

Holdsworth (1952) isolated an oligosaccharide with a molecular weight of about 1200 from cell walls of *C. diphtheriae*. This contained six sugar residues; three arabinose, two galactose and one mannose. On treatment of the oligosaccharide with periodate, however, galactose disappeared while arabinose and mannose were apparently unaffected. The difference between his findings and the present results may indicate that galactose is in some way concerned with the attachment of the oligosaccharide in the cell wall complex, and may also suggest that mannose is concerned in the immunological specificity of the material.

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Separations of Basic Amino Acids by Ionophoresis in Filter Paper. By H. HARRIS and F. L. WARREN. (*Department of Biochemistry, London Hospital Medical College, E.* 1)

A satisfactory separation of the basic amino acids in amino acid mixtures is often difficult to achieve by conventional methods of paper chromatography. A number of useful unidimensional separations can however be obtained by ionophoresis in filter paper, and these have proved valuable as an adjunct to chromatography in the identification of basic amino acids present in biological fluids.

The ionophoresis has been carried out in the apparatus described by Flynn & de Mayo (1951). This apparatus takes papers 38 cm. in length and the width can be varied up to 40 cm. according to the number of different amino acid solutions one wishes to run in parallel. No. 1 Whatman filter paper has been used throughout.

The amino acid solution is applied to the apex of the filter paper and the buffer allowed to ascend evenly from the buffer compartments on each side so that no displacement of the amino acids takes place. A constant potential of 185v applied for 16–17 hr. has been found to give satisfactory results.

At pH 11·5 (0·05M-Na_2CO_3) lysine and arginine migrate in that order towards the anode and form compact spots completely separated from each other and from other naturally occurring amino acids. Ornithine moves more rapidly towards the anode than lysine, but cannot be separated from proline or γ-aminobutyric acid which move at about the same speed. Histidine, methylhistidine, β-alanine, β-

amino*iso*butyric acid, the neutral amino acids with the exception of proline, and the acidic amino acids all move more rapidly than ornithine and most of them leave the paper and enter the anode compartment.

At pH 8·6 (0·05M sodium diethylbarbiturate/0·01M diethylbarbituric acid), lysine, arginine and ornithine migrate off the paper into the cathode compartment. Methylhistidine and histidine, however, appear to be negatively charged and migrate towards the anode, methylhistidine moving more rapidly and becoming separated from histidine. At this pH methylhistidine can be conveniently separated from anserine (β-alanylmethylhistidine).

β-Amino*iso*butyric acid and β-alanine are somewhat more basic than the α-amino acids. They can be conveniently separated from them at pH 4·0 (0·05M potassium hydrogen phthalate). Under these conditions lysine, arginine, ornithine, methylhistidine, histidine and γ-aminobutyric acid move off the paper into the cathode compartment. β-Alanine and β-amino*iso*butyric acid move in that order towards the cathode but remain on the paper. The neutral α-amino acids carry no appreciable net charge but are displaced slightly towards the cathode by the endosmotic flow. The acidic amino acids migrate towards the anode.

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Excretion of Amino Acids in Cystinurics. By H. Harris, Ursula Mittwoch, Elizabeth B. Robson and F. L. Warren. (*Galton Laboratory, University College, London, and Department of Biochemistry, London Hospital Medical College*)

The urine of patients who form cystine calculi in the renal tract contains not only high concentrations of cystine, but also of lysine, arginine and ornithine (Dent & Rose, 1951; Stein, 1951). Among the close relatives of such patients many individuals are found who while excreting abnormal amounts of cystine nevertheless do not form cystine calculi, presumably because the cystine concentration in the urine is below the saturation point (Harris & Warren, 1953).

The cystine and lysine contents of urine specimens from twenty-eight patients with cystine stone formation and 119 of their close relatives have been estimated. The cystine was estimated polarographically and the lysine microbiologically. In many samples the arginine has also been estimated microbiologically. The creatinine content of the samples was determined and the quantities of cystine, lysine, and arginine present per g. of creatinine have been used as indices of the excretion of these amino acids. In all cases the urinary amino acids have been examined qualitatively by chromatography.

Considerable variation occurs between individuals in the excretion of these amino acids. Cystine outputs measured in this way vary from normal values of 25–60 up to 700 mg./g. of creatinine and lysine outputs from normal values of 0–40 up to 1500 mg./g. of creatinine. Determinations on several samples from particular individuals over periods of 6 months to 2 years indicated that each person had a fairly characteristic level of excretion of these amino acids.

Over the whole range there is a high correlation between cystine and lysine output ($r = 0{\cdot}95$).

Abnormally high arginine excretion was only consistently found when the cystine and lysine values were in their upper ranges. Below these levels, although cystine and lysine might still be well above those occurring in normals, the arginine excretion was either normal or at most very slightly above normal. The ornithine excretion may well follow the same pattern. Other amino acids are not excreted in increased amounts in either the cystinuric patients or their relatives.

The results imply a close connection between the processes involved in the excretion of cystine, lysine, arginine and ornithine. It has been suggested that in cystinuric patients there is a specific failure in the renal tubular reabsorption of these four amino acids from the glomerular filtrate (Dent & Rose, 1951; Harris, Fowler & Warren, 1952; Dent & Joron, 1954; Dent, Senior & Walshe, 1954). If this is so, the failure in reabsorption is probably complete or nearly complete in the patients with stone formation and very high excretion of these amino acids, but can only be partial in their relatives with only moderate degrees of abnormal excretion. Here the impairment is presumably confined to cystine and lysine while arginine reabsorption is relatively much less disturbed.

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Carboxylic Acids and Carotenogenesis in *Phycomyces blakesleeanus*. By J. FRIEND, T. W. GOODWIN and L. A. GRIFFITHS. (*Department of Biochemistry, University of Liverpool*)

Schopfer & Grob (1950) found that on a medium containing lactate as the sole carbon source, *Phycomyces blakesleeanus* grew only slightly and produced no carotene; addition of acetate (1% w/v) to this medium stimulated both growth and carotenogenesis. They found later that lactate was not necessary and that acetate alone was adequate. These observations on media containing lactate and acetate have now generally been confirmed, although small amounts of carotene are formed on the 'lactate-only' medium. A 'pyruvate-only' medium gives results similar to those obtained with the 'lactate-only' medium. The usual method of determining dry weight in *P. blakesleeanus* (Garton, Goodwin & Lijinsky, 1951) cannot be used with cultures grown on these media because as the pH increases from 6·5 to 9·0 during growth, phosphates are thrown down which become embedded in the mycelium. Unless the mycelia are washed with dilute acid, dry-weight determinations will be much too high. Acid washing of mycelia grown on a glucose medium (pH 5·5–4·0) does not cause any appreciable loss of weight.

Addition of yeast extract (0·1%) to an acetate medium stimulates growth and lipid production by 3–4 times and β-carotene synthesis to a greater extent. The carotene concentration on a yeast-acetate medium approaches that on a glucose medium, but the yield is much less.

Stimulation of growth and carotenogenesis by yeast extract is also observed on the lactate and pyruvate media but not on the glucose medium.

Substitution of ammonium acetate as the N sources in 'acetate media' by NH_4NO_3 or asparagine did not stimulate β-carotene synthesis, but some stimulation was observed with leucine.

The report (Leonian & Lilly, 1940) that the addition of various carboxylic acids at a level of 0·1% (w/v) to a glucose (2·5% w/v)/NH_4NO_3 medium stimulated growth of *P. blakesleeanus* has been confirmed using acetate and succinate. Carotenogenesis is stimulated to the same extent. Addition of succinate or other dicarboxylic acids at the same level (0·1% w/v) to the 'acetate' medium did not stimulate either growth or carotenogenesis.

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The Concentration of the Cholinesterase Activity of Adrenal Medulla in the Microsome Fraction. By P. HAGEN. (*Department of Pharmacology, University of Oxford*)

That the cholinesterase activity of adrenal gland is almost entirely medullary was shown for ox, pig and rabbit by Antopol & Glick (1940) and for man by Langemann (1942). Ord & Thompson (1950) have shown that the adrenal cholinesterase in the rat is largely acetylcholinesterase, and Langemann (1951) has shown that this also holds for the ox. By differential centrifugation it has now been possible to determine the intracellular localization of this enzyme.

Dispersions of ox adrenal medulla in ice-cold isotonic (0·3 M) sucrose were prepared and subjected to centrifugation, yielding (1) a first supernatant free from nuclei, from which were derived (2) a 'large granule' fraction and (3) a fraction containing the rest of the cytoplasm. Centrifugation of the last fraction (3) at 100,000 *g* for 30 min. yielded (4) a microsome fraction and (5) a non-particulate final supernatant.

The acetylcholinesterase activity of these fractions was determined by the technique of Ammon (1933). The results of a typical experiment were as follows (values given are micromoles of acetylcholine hydrolysed per mg. protein nitrogen per hour). First supernatant 6·67, 'large granule' fraction 4·65, microsome fraction 30·4, and final supernatant 4·86. That a clear separation of fractions was obtained is shown by the fact that determinations of the succinoxidase activity of the same fractions gave the following values (as micromoles succinate oxidized per mg. protein nitrogen per hour) first supernatant 22, 'large granule' fraction 51·2, microsome fraction 6·0, and final supernatant 0·0. We have repeated this experiment four times and have found the same intracellular distribution of the cholinesterase activity.

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The Activation of Hydrogen by Bacteria. By A. Couper, D. D. Eley and A. Hayward. (*Department of Chemistry, University of Bristol*)

The fixation of hydrogen by bacteria (Stephenson & Stickland, 1931) is associated with the *ortho-para* conversion of hydrogen and deuterium (Farkas, 1936) and the exchange of deuterium with water (Hobermann & Rittenberg, 1943). The activation energy of the *para*-hydrogen conversion was found to be 10·0 kcal. in the presence of suspensions of *Proteus vulgaris* and *Escherichia coli* containing $3{\cdot}8 \times 10^8$ cells per ml., with lower values in more concentrated suspensions, or for the most active cultures at higher temperatures, when diffusion processes became rate-limiting. Reducing the cell concentration was less effective in eliminating the low activation energy process, than raising the hydrogen pressure, a value of 15·0 kcal. being obtained for the exchange of deuterium with water (Farkas & Fischer, 1947). The conversion of hydrogen by paramagnetic ions in solution proceeded at a greater rate than in suspensions of bacteria, without limitation of the rate by diffusion.

After allowing for a small difference in activation energy between the *para*-hydrogen conversion and deuterium/water exchange in a number of cultures, the probability of the former reaction was about twice that of the latter. It was concluded that the probability of an active hydrogen atom combining with another was about equal to that of its reacting with a water molecule.

The rate of hydrogenation of methylene blue was about one-tenth of the rate of exchange reactions, in agreement with the values 0·5–0·1 for the ratio of the rates of hydrogenation of fumarate, and of deuterium exchange in *Proteus* suspensions (Farkas & Fischer, 1947).

Dehydrated bacteria were completely inactive, but on introducing water vapour, the activity revived, and when about three times the dry weight of bacteria had been added, a maximum velocity was reached, only slightly less than that observed in suspension. The rate of *para*-hydrogen conversion was again greater than that of deuterium exchange. Hydration by pure heavy water inhibited the exchange of hydrogen, as found for other enzymes (Shibata & Watanabe, 1944), and on replacing deuterium oxide by water, the faster exchange was observed, although some permanent inactivation had occurred. When partially inactivated by heat, the same amount of water was required to give the reduced maximum velocity, but when the bacteria were inactivated by heavy water, a quantity of water proportional to the new velocity was required.

The activation by water will be discussed in terms of the swelling of the cell membrane so as to expose enzymes held in it.

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Electrophoretic Separation of Plasma Lipoproteins. By D. W. Barritt (introduced by R. J. Brocklehurst). (*Department of Medicine, University of Bristol*)

60 μl. of serum are fractioned by electrophoresis in a barbitone buffer at a potential of 300 v for 5 hr. The paper is stained with fresh Sudan Black in 50% (v/v) industrial spirit solution. The lipoproteins are found in two main fractions corresponding to the α- and the β-globulin protein fractions. The paper is cut into 2 cm. strips and the dye is eluted in acetic acid solution. The solutions are read off on a spectrophotometer at a wavelength of 590 mμ.

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The antithrombin activity of human plasma. By J. W. LYTTLETON

The antithrombin activity of heparin. By J. W. LYTTLETON

The identification of propionylcholine as a constituent of ox spleen. By J. E. GARDINER and V. P. WHITTAKER

Appendix—The identification of spleen propionylcholine by infrared microspectroscopy. By D. L. WOOD

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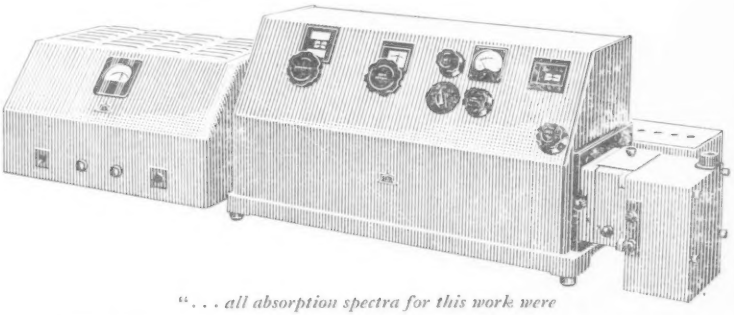

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